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RAPID COMMUNICATION / COMMUNICATION RAPIDE

Sudden appearance of cysts and ellobiopsid parasites on zooplankton in a Michigan lake: a potential explanation of tumor-like anomalies

Thomas B. Bridgeman, Gretchen Messick, and Henry A. Vanderploeg

Abstract: Cysts on calanoid and cyclopoid copepods, previously reported as tumor-like anomalies (TLAs) in Lake Michigan and Europe, appeared briefly in Patterson Lake, a small Michigan inland lake. Cysts were rare (4% maximum) in samples collected on September 11, 1999, but appeared with high frequency on calanoid adults (49%) and cyclopoid nauplii (73%) in samples collected on October 16. By October 30, cysts were again rare (0.4% maximum). Cysts most commonly appeared on the lateral surface of the animal at the articulation of the 1st and 2nd prosomal segments and often consisted of herniated copepod tissues. Transparent, pyriform cysts co-occurred in low frequency with other types of cysts and are believed to be the trophomeres and gonomeres of ellobiopsid parasites. Histologic manifestations of cysts were diverse; herniations consisted of acellular yolk-like material and apparent host tissue, while cysts thought to be *Ellobiopsis* contained cells with different degrees of nuclear staining and unusual spherical bodies. Hernias were experimentally induced on live calanoid copepods by piercing the carapace with a fine needle, suggesting that ellobiopsid parasites may cause the hernias by puncturing the carapace of their hosts. Ellobiopsid parasites are common on marine crustacean zooplankton but have been recorded only once before in freshwater.

Résumé : Des kystes affectant les copépodes calanoïdes et cyclopoïdes, déjà signalés comme anomalies pseudotumorales dans le lac Michigan et en Europe, ont fait une brève apparition dans le lac Patterson, petit lac intérieur du Michigan. Les kystes étaient rares (4% maximum) dans les échantillons recueillis le 11 septembre 1999, mais apparaissaient avec une forte fréquence chez les adultes de calanoïdes (49%) et les nauplius de cyclopoïdes (73%) dans les échantillons prélevés le 16 octobre. Le 30 octobre, les kystes étaient redevenus rares (0,4% maximum). Les kystes apparaissaient le plus souvent sur la surface latérale de l'animal, à l'articulation du 1er et du 2e segment prosomal, et consistaient souvent en hernies des tissus des copépodes. Des kystes transparents et piriformes, présents à faible fréquence avec d'autres types de kystes, pourraient être des trophomères et des gonomères d'ellobiopsidés parasites. Les manifestations histologiques des kystes étaient diverses; les hernies consistaient en matière acellulaire de type vitellin et en tissu apparent de l'hôte, tandis que les kystes attribués à *Ellobiopsis* contenaient des cellules présentant différents degrés de coloration du noyau et des corps sphériques inhabituels. Nous avons expérimentalement induit des hernies chez des copépodes calanoïdes vivants en perçant la carapace avec une aiguille fine, ce qui permet de penser que les ellobiopsidés parasites peuvent causer les hernies en perforant la carapace de leurs hôtes. Les ellobiopsidés parasites se retrouvent couramment sur les crustacés du zooplancton marin, mais n'avaient jusqu'ici été signalés qu'une fois en eau douce.

[Traduit par la Rédaction]

Introduction

Recent reports of tumor-like anomalies (TLAs) on crustacean zooplankton in Lake Michigan (Omair et al. 1999) have aroused interest in the nature and distribution of the abnormalities and their effects on zooplankton populations. TLAs found on copepods in Lake Michigan and several other Laurentian Great Lakes (M. Tuchman, EPA Great Lakes National Program Office, Chicago, Ill., personal communication) are similar in appearance to "cysts" reported from Lake

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T.B. Bridgeman.[1] Cooperative Institute for Lake Ecosystems Research and Department of Biology, University of Michigan, 830 North University Avenue, Ann Arbor, MI 48109-1048, U.S.A.
G. Messick. National Ocean Service, NOAA, Center for Coastal Environmental Health and Biomolecular Research Cooperative Oxford Laboratory, 904 South Morris Street, Oxford, MD 21654-9724, U.S.A.
H.A. Vanderploeg. Great Lakes Environmental Research Laboratory (GLERL) / NOAA, 2205 Commonwealth Blvd., Ann Arbor, MI 48105, U.S.A.

[1]Author to whom all correspondence should be addressed. e-mail: tbbridge@umich.edu

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Maggiore, Italy (Manca et al. 1996), and the "protruded protoplasmatic masses" described from copepods in the Mediterranean Sea (Crisafi and Crescenti 1975). To avoid the suggestion of neoplasia associated with the word "tumor," we use "cyst" to describe the abnormalities in general and "hernia" for those consisting of extruded internal tissues.

We report an episodic occurrence of cysts among the copepods of a small inland lake, including a brief histologic description of the cysts. We present evidence that some types of cysts are ellobiopsid parasites and that many other cysts are hernias potentially caused by damage to the carapace.

Materials and Methods

Site description

Patterson Lake and a second lake sampled for comparison (Third Sister Lake) are located within the Huron River watershed in southeastern Michigan. Patterson Lake (42°26.18′N, 84°01.40′W) is similar in area (0.62 km^2) and depth (z_{max} = 20 m) to other lakes in the region. The lake is used for recreation and has a wooded shoreline except for approximately 100 residences. During the autumn sampling period, the lake was stratified and the hypolimnion was anoxic. Third Sister Lake (area = 0.68 km^2, z_{max} = 17.5 m) lies 25 km southeast of Patterson Lake (42°16.54′N, 83°48.35′W) in an area characterized by light industry and residential development.

Methods

Zooplankton samples were collected at Patterson Lake on September 11, October 16, and October 30, 1999, and February 2 and April 18, 2000, from a location about 100 m from the northeast shore (z = 12 m) by vertical hauls of a 153-μm-mesh plankton net with a 30-cm-diameter opening. The September sample was collected by hauling from 6 m (1 m below the oxycline, <1 $mg \cdot L^{-1}$ dissolved oxygen) to the surface and was preserved in 4% sugar formalin. Because the mixed layer deepened in October, all subsequent hauls were made from within 1 m of the bottom.

The October 16 sample was examined immediately after collection at which time cysts were first noted. This observation prompted subsequent collections on October 20 (Third Sister Lake, 12 m) and October 30 (Patterson Lake) and the examination of the September 11 sample from Patterson Lake. On October 16, 1 mL of 0.1% chloral hydrate and club soda were added before preservation to prevent the egestion of gut contents. The same procedure was followed in subsequent collections to maintain consistency.

To determine if our collection and preservation techniques induced the formation of cysts, four vertical tows were made at Patterson Lake on April 18. The first two tows were preserved as before and the 3rd and 4th tows were transferred to 5-L bottles containing lake water and transported live to the laboratory. Batches of live animals were transferred to a counting chamber and scanned for cysts under a dissecting microscope until 200 nauplii and 100 calanoid copepods had been examined. The live sample was then split, with half preserved as previously described and the second half subjected to approximately double the amount of chloral hydrate, club soda, and sugar formalin (final concentration 10%). Both laboratory- and field-preserved samples were then scanned for cysts under a dissecting microscope.

In an effort to induce cysts in live copepods, individual adult and immature calanoid copepods were isolated in drops of lake water. A fine hypodermic needle (insulin 28G) was used to pierce the carapace on the dorsal side. Nauplii were not used because of their small size.

Photographs were made using dissecting and compound microscopes equipped with a digital camera. Sizes of cysts were measured from the images using a PC with analytical software (SPOT V2.2, Diagnostic Instruments, Sterling Heights, Mich.).

Copepods processed for histology were transferred from 4% sugar formalin into 70% ethanol. Copepods were photographed individually to record gross morphology and coded for tracking for histologic manifestations. Specimens were dehydrated, infiltrated with paraffin, embedded, sectioned at 4–6 μm thickness and stained with Meyer's hematoxylin and eosin.

Results

Cysts occurred only on copepod species. Although cladoceran species, including *Daphnia retrocurva*, *Daphnia pulicaria*, *Bosmina longirostris*, *Ceriodaphnia* sp., and *Leptodora kindti*, were common in Patterson Lake, no cysts were observed on them. Cyclopoid copepods included *Diacyclops thomasi*, *Acanthocyclops* sp., *Tropocyclops prasinus*, and *Mesocyclops* sp. All adult calanoid copepods were *Skistodiaptomus oregonensis* in the autumn samples. The abundance of copepods and frequencies of cysts in Patterson Lake and Third Sister Lake are given in Table 1. Life stages are divided into three major groups: nauplii (larvae), copepodids (juveniles), and adults.

High frequencies of cysts occurred only in the October 16 sample from Patterson Lake. The two most acutely affected groups were the naupliar larvae of cyclopoid copepods and the adults of *S. oregonensis*. In the latter group, the frequency of cysts was equal for males and females (chi-square, $p > 0.50$). Juvenile stages of both cyclopoid and calanoid species were moderately affected, but no cysts were found on any adult cyclopoid. The zooplankton community of Third Sister Lake was similar in composition to that of Patterson Lake, but on October 20, cysts were rare in Third Sister Lake. No cysts were found in preserved samples from Patterson Lake in February and April 2000. Cysts were not found on live zooplankton collected in April and did not appear after the animals were preserved. Re-examination of all samples over several weeks did not reveal any obvious change in the frequency or appearance of cysts as the result of preservation in formalin.

Cysts most commonly appeared on the lateral surface of adult *S. oregonensis* at the articulation of the 1st and 2nd prosomal segments (Fig. 1*a*). Cysts ranged from small (7×10^{-4} mm^2 dorsal surface area) to large (5×10^{-2} mm^2, Fig. 1*b*) but were typically intermediate in size ($3–11 \times 10^{-3}$ mm^2, Fig. 1*a*) and rough-edged in appearance (Fig. 1*a*). Smooth, rounded cysts (Fig. 1*b*) and elongated, transparent cysts (Figs. 1*c*, 1g, and 1*i*) were present but rare (<1%).

Cysts had diverse histologic characteristics that are currently under detailed investigation. Some cysts were composed of a largely acellular mass, containing yolk-like material and a margin of host-derived cells defining the outer periphery. Many cysts appeared as herniated host tissue and often demonstrated communication between the cyst and the host (Fig. 1*f*). Other cysts consisted of masses of cells appearing to be host hemocytes that formed protrusions on the external surface of the copepod.

Elongated and pyriform transparent cysts closely resembled ellobiopsid parasites. These cysts contained granular material and numerous round bodies in various stages of spore formation. Round bodies were observed with no apparent nuclear material (Figs. 1*d* and 1*j*), numerous small

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Table 1. Occurrence of cysts (all types) on copepods in Patterson Lake and Third Sister Lake.

	Patterson Lake									Third Sister	
	Sept. 11			Oct. 16			Oct. 30			Oct. 20	
	Ind.·L^{-1}	%	*N*	Ind.·L^{-1}	%	*N*	Ind.·L^{-1}	%	*N*	%	*N*
Cyclopoida											
Nauplii	2.6	3	119	3.1	73	55	1.4	0	27	8	61
Copepodids	15.1	0	618	11.8	28	103	14.7	0.4	231	0	200
Adults	4.2	0	203	1.7	0	39	0.4	0	14	0	43
Calanoida											
Nauplii	1.7	1	95	11.1	21	238	13.3	3	212	0	36
Copepodids	6.3	0	340	9.3	17	170	23.0	3	259	0	61
Adult *S. oregonensis*	1.9	4	72	1.1	49	63	3.9	1	79	0	43

Note: Ind., individuals; %, percentage of *N* individuals in which cysts occurred.

nuclei (Fig. 1*j*), nuclei of various sizes (Fig. 1*j*,), or undergoing cleavage (Fig. 1*j*). Round bodies varied in size and were occasionally present within the body of the copepod as well.

Piercing the carapaces of live adult calanoid copepods with a fine needle produced immediate hernias, often of gonadal tissue, that appeared to be identical with many of the cysts observed in the October 16 sample (Figs. 1*e* and 1*f*). Piercing immature calanoid copepods resulted only occasionally in the extrusion of internal tissues. Hernias could not be induced on formalin-preserved specimens.

Discussion

Live collections of zooplankton are rarely examined closely, and to date, all reports of TLAs or cysts have come from preserved samples. The absence of observations of cysts on live zooplankton raises a question of whether cysts might be an artifact of collection and preservation. Although we did not find cysts on live copepods, our normal preservation procedure or doubling the concentration of preservative did not induce cysts. None of the copepods or cladocerans in our preserved collections had carapace ballooning or loss of eggs that might result from the osmotic shock of death and preservation. Although piercing the carapace of copepods induced hernias very similar to those found in our collections, we do not believe we could have injured large numbers of animals in this way by collection alone.

Male and female calanoid copepods with mature gonads appeared to have an internal hydrostatic pressure or turgor that caused gonad and other tissues to extrude through the punctures. Copepodids had less turgor; therefore, piercing them produced hernias less often. Differences in turgor between mature and immature calanoid copepods may explain the higher frequency of hernias on adult calanoids than on calanoid copepodids observed in Patterson Lake on October 16, 1999.

In samples where hernias occurred, a few elongate and pyriform transparent cysts were also found. The transparent cysts were very similar in appearance to ellobiopsid parasites reported on calanoid copepods from Lake Midmar in South Africa (Rayner and King 1986).

The family Ellobiopsidae is a small, heterogeneous group of protist parasites on marine crustacean zooplankton thought to be allied with the dinoflagellates (Theodorides 1989). The life histories of the ellobiopsids are diverse and only partially understood. The genus *Thalassomyces* develops internally and then erupts through the carapace of the host (Mauchline 1966), while *Ellobiopsis* is believed to attack the carapace externally (Jepps 1937). A common feature of many ellobiopsids is the appearance on the host of a stalked or ellipsoid trophomere bearing a fruiting gonomere at the distal end. The transparent cysts in Figs. 1*c*, 1*g*, and 1*i* bear a strong resemblance to trophomeres of the genus *Ellobiopsis* illustrated in Boschma (1959) and Rayner and King (1986). The pyriform cysts in Figs. 1*h* and 1*i* have knurled distal ends and enclose a darker interior body similar to the cysts in Rayner and King (1986). The elongated cysts in Figs. 1*c* and 1*g* are more similar to the descriptions of mature *Ellobiopsis* by Jepps (1937), Boschma (1959), and Theodorides (1989), suggesting that the pyriform cysts are immature versions. The specimen in Fig. 1*g* bears cysts of both shapes. The internal structure of *Ellobiopsis* is generally granular, with no large nuclear structures (Jepps 1937). At times, however, small nuclei can be observed (Jepps 1937) and the granular material may occur in spherical globules (Boschma 1959).

Ellobiopsid parasites are fairly common in marine environments but Lake Midmar (Rayner and King 1986) is the only freshwater report of ellobiopsids. In some reports of TLAs or cysts, however, ellobiopsid parasites may have gone unrecognized. Among other cysts, Crisafi and Crescenti (1975) reported copepods with elongate, transparent cysts similar to our Fig. 1c. Photographs of TLAs on immature calanoid copepods from the Baltic Sea (T. Shchuka, Institute of Global Climate and Ecology of the Russian Academy of Science and Rosgidromet, Moscow, personal communication) include forms that are probably ellobiopsid parasites. Although Omair et al. (1999) did not report finding crustaceans with elongated, transparent cysts in Lake Michigan, several have recently been found. One specimen is clearly *Ellobiopsis*, having a distinct trophomere and gonomere separated by a constriction and septum (J. Cavaletto, GLERL, Ann Arbor, Mich., personal communication). TLAs with stalked attachments to the mouthparts or antennae of *Limnocalanus* in Lake Michigan (Omair et al. 1999) match the description of *Ellobiopsis* occurring on *Calanus* in the Clyde Sea (Jepps 1937).

We suggest that ellobiopsid parasites are responsible for many of the cysts found in Patterson Lake and TLAs reported elsewhere. The hernias on Patterson Lake copepods likely resulted from punctures in the carapace possibly

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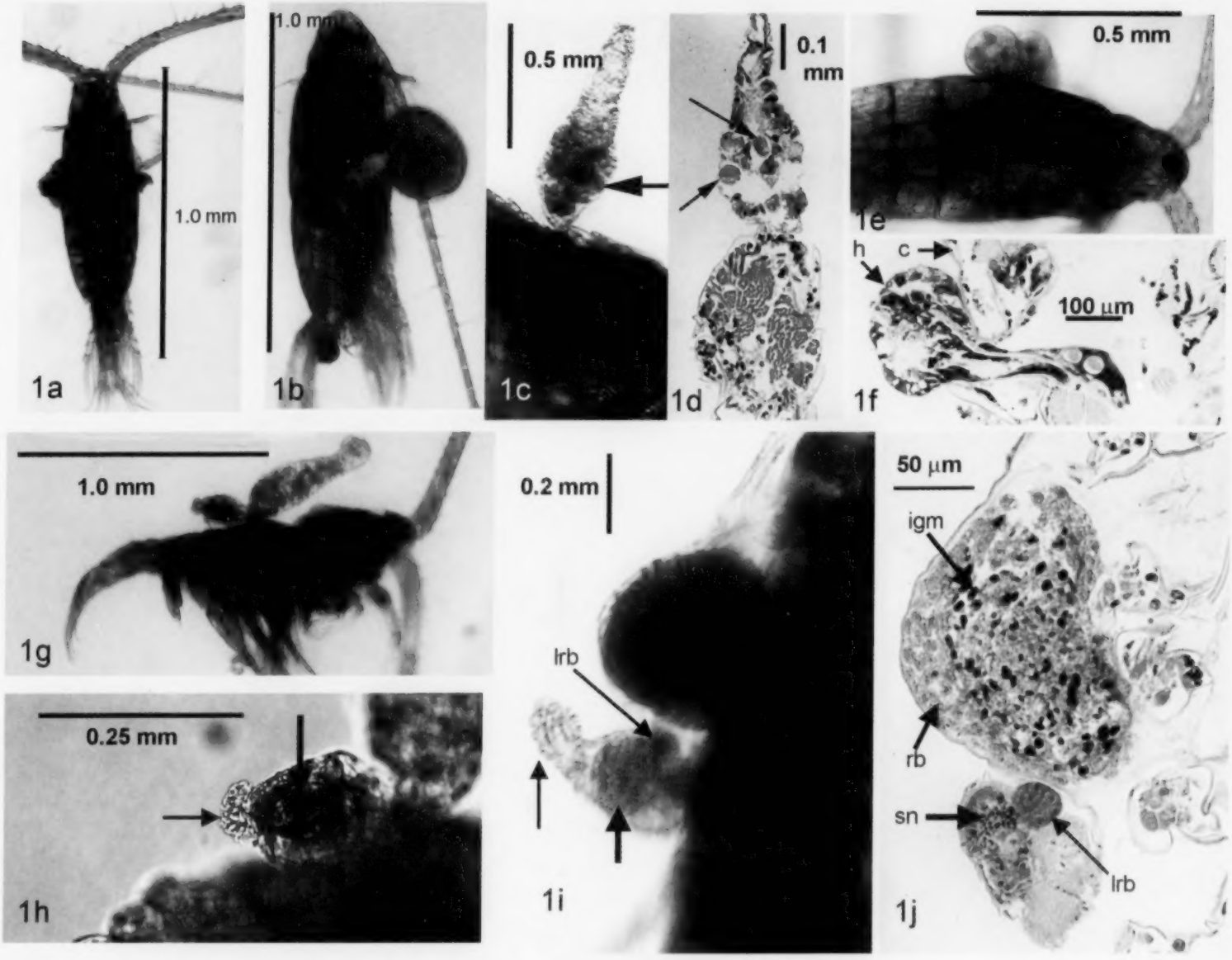

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Fig. 1. Gross morphology and histological sections of copepods with cysts and hernias from Patterson Lake, Michigan. (*a*) Adult *Skistodiaptomus oregonensis* with hernias at the articulation of 1st and 2nd prosomal segments. (*b*) *S. oregonensis* with large cyst similar to tumor-like anomalies reported from Lake Michigan (Omair et al. 1999). (*c*) *S. oregonensis* bearing elongated, transparent cyst of the type thought to be an ellobiopsid parasite. Note round sporulation bodies (arrow). (*d*) Histological section of specimen in Fig. 1*c*; round bodies are indicated by arrows. (*e*) Live *S. oregonensis* with hernia induced by piercing the carapace with a hypodermic needle. (*f*) Histological section of a hernia (h) protruding from a break in the carapace (c). (*g*) External view of pyriform and elongated cysts thought to be ellobiopsid parasites. (*h*) Enlarged view of pyriform cyst. Note knurled distal end (small arrow) and ovoid interior body (larger arrow) characteristic of ellobiopsid parasite. (*i*) Another example of a pyriform cyst with knurled end (small arrow), ovoid interior body (larger arrow), and large round body (lrb). Large, dark cyst above may be a different stage of the parasite. (*j*) Histological section of cysts shown in Fig. 1*i*. Note various stages of spore development: round bodies (rb) with no nuclear material along outer periphery, nuclei of various sizes among inner granular material (igm), abundance of small nuclei (sn) within the larger ovoid body. Large round body (lrb) is undergoing cleavage and may become the next ovoid body.

caused by parasites. Although the mature parasite was found only on adult and nearly adult calanoid copepods, the co-occurrence in copepod populations of parasites and herniated individuals in Patterson Lake, Lake Michigan (J. Cavaletto, GLERL, Ann Arbor, Mich., personal communication), and the Baltic Sea (T. Shchuka, Institute of Global Climate and Ecology of the Russian Academy of Science and Rosgidromet, Moscow, personal communication) suggests a connection between parasites and hernias. Recognizable ellobiopsid parasites were rare compared with other types of cysts in Patterson Lake, which may explain why they have been overlooked in previous reports of cysts and TLAs.

The sporadic nature of ellobiopsid infestations has been reported by others (Rayner and King 1986, Al-Yamani and Fahmi 1995). In Patterson Lake, cysts on copepods appeared with high frequency in mid-October only, suggesting that the cysts developed within a few weeks. Although cysts were rare in late October, it is unlikely that they were shed by molting because most cysts are deeply anchored through a small hole in the carapace (Hoffman and Yancey 1966). It is more likely that increased mortality resulting from infection, starvation, inability to molt, or increased susceptibility to predation may explain the absence of animals bearing cysts in late October.

Nearly opposite patterns of infections were observed between calanoid and cyclopoid species. In calanoids, cysts were most frequent in adults. Conversely, cysts were absent among adult cyclopoids but occurred frequently on nauplii. Cyclopoid nauplii were also highly affected in several Russian lakes where cyst frequencies as high as 69% were observed (Silina and Khudolei 1994). The absence of cysts on adult cyclopoid copepods in all reports of cysts, TLAs, or ellobiopsid parasites suggests that although cyclopoid juveniles may be susceptible, adult cyclopoids are not suitable hosts for the parasites.

The effect of cysts on individuals or populations is difficult to determine without more frequent sampling intervals or observations of infected live animals. Some species of ellobiopsid parasites are thought to sterilize the host or reduce gonad development (Ramirez and Dato 1989). In Patterson Lake, egg-bearing females were uncommon, but some *S. Oregonensis* carried a few eggs as well as ellobiopsid cysts. *Ellobiopsis* was also thought to have played a role in the replacement of *Tropodiaptomus spectabilis* by a competitor, *Metadiaptomus transvaalensis*, which was immune to the parasites (Rayner and King 1986).

Although this is the first record of cysts on copepods from a small lake in North America, and only the second record of ellobiopsid parasites occurring in fresh water, it is difficult to know whether this phenomenon is new. At low frequencies, cysts would be easily missed in a standard analysis of plankton samples. Even when cysts are common, those having the characteristics of ellobiopsid parasites are rare. If, as in Patterson Lake, high frequencies of cysts are present for only a few days or weeks, even weekly collections could miss the period in which cysts would be frequent enough to become obvious.

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An evaluation of alternative procedures using the Bou–Rouch method for sampling hyporheic invertebrates

Gary W. Hunt and Emily H. Stanley

Abstract: This study assesses the influence of well design, pumping rate, and sample volume on hyporheic invertebrate density and richness estimates in three streams differing in sediment composition. A comparison of five well designs, i.e., temporary wells with no pores and with 4-, 6-, and 8-mm pores along the lower 15 cm and permanent wells with 6-mm pores, revealed no significant differences in either total invertebrate densities or taxon richness. Pumping rates, i.e., 1.5 versus 4 L·min^{-1}, significantly affected density estimates at two streams and taxon richness at one of the three streams, shown by higher values at the faster pumping rate. A comparison of five sample volume sizes, i.e., 0.5, 1.0, 1.5, 2.0, and 2.5 L, indicated that smaller sample volumes produced significantly higher estimates of density, while larger sample volumes gave significantly higher estimates of taxon richness. These results underscore the importance of maintaining consistent pumping rates and sample volume sizes throughout the course of a study.

Résumé : Notre étude évalue l'influence de la conception du puits, du taux de pompage et du volume échantillonné sur les estimations de la densité et de la richesse des invertébrés de la zone hyporhéique dans trois cours d'eau différant par la composition des sédiments. La comparaison de cinq puits de conception différente (puits temporaires sans pores, à pores de 4, 6 et 8 mm le long de la bande inférieure de 15 cm, et puits permanents à pores de 6 mm) n'a fait ressortir aucune différence significative dans la densité totale des invertébrés ni dans la richesse taxonomique. Les taux de pompage (1,5 contre 4 L·min^{-1}) avaient un effet significatif sur les estimations de la densité dans deux cours d'eau, et sur celles de la richesse taxonomique dans un des trois cours d'eau, les valeurs étant plus élevées quand le taux de pompage était plus fort. La comparaison de cinq volumes d'échantillonnage (0,5, 1,0, 1,5, 2,0 et 2,5 L) a indiqué que les échantillons à volume plus faible fournissaient des estimations significativement plus élevées de la densité, tandis que les échantillons à volume plus grand fournissaient des estimations significativement plus élevées de la richesse taxonomique. Ces résultats font ressortir l'importance du maintien d'un taux de pompage et d'un volume d'échantillon constants tout au long d'une étude.

[Traduit par la Rédaction]

Introduction

Recent advances in understanding the hyporheic zone have added significantly to stream ecology (Dahm and Valett 1996). However, examination of subsurface invertebrate communities has lagged behind research emphasizing ecosystem-level processes (Valett et al. 1993; Stanley and Jones 2000). Reasons for this lag include the large number of previously undescribed species inhabiting the hyporheic zone and problems associated with obtaining reliable quantitative data describing the abundance of organisms occurring in this habitat (Fraser and Williams 1997). There is an urgent need to assess existing sampling methods across a range of stream types before substantive progress can be made in understanding the ecology of hyporheic invertebrate communities (Hakenkamp and Palmer 2000).

Invertebrate sampling methods range from the simple approach of collecting individuals from pits excavated on stream banks or instream bars by filtering inflowing water (i.e., the Karaman–Chappuis method (Chappuis 1942)) to complex coring techniques. While core sampling appears to be the most reliable quantitative method (Williams and Hynes 1974), it is unfortunately limited in depth and to streams with sandy sediments (Palmer and Strayer 1996). Alternative approaches that have been used include freeze coring (Stocker and Williams 1972; Hynes 1974; Bretschko 1985) and the use of artificial substrates (Tabacchi 1990). However, these methods can be time consuming, labor intensive, and expensive.

The method perhaps most often used to collect both chemical and invertebrate samples consists of pumping hyporheic water from specific depths in the streambed using either temporarily or permanently installed standpipe wells (Palmer and Strayer 1996). This technique, originally described by Bou and Rouch (1967), requires relatively little labor and only inexpensive equipment. However, specific concerns have been raised as to the accuracy of data derived from this method. Standpipe wells may be selective for specific groups of organisms, depending on the volume sampled (Danielopol 1976) and the filtering effect of the substrate (Williams 1984). Fraser and Williams (1997) reported that well samples underestimated larger animals, i.e., insect larvae, that are able to grasp onto substrate particles and thus

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G.W. Hunt.[1] Division of Math and Science, Tulsa Community College, Tulsa, OK 74119-2095, U.S.A.
E.H. Stanley. Center for Limnology, University of Wisconsin, Madison, WI 53706-1492, U.S.A.

[1]Author to whom all correspondence should be addressed.
e-mail: ghunt@tulsa.cc.ok.us

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Table 1. Site attributes of the the study streams used to evaluate hyporheic sampling methods.

	Baron Fork	Rock Creek	Sager Creek
Latitude	35°55′	34°35′	36°12′
Longitude	95°51′	96°58′	94°35′
Sampling date	18 July 1998	5 July 1998	27 June 1998
Depth (cm)	21–30	4–20	8–13
Width (m)	36	8	20
Water temperature (°C)	25	23	26
Sediments composition (% by weight)			
Fine sand (<0.25 mm)	3.3	5.5	3.5
Medium sand (0.25–0.5 mm)	3.5	18.1	1.8
Coarse sand (0.5–2.0 mm)	12.7	60.1	5.0
Very coarse sand (2.0–6.3 mm)	15.5	14.8	13.4
Gravel and (or) cobbles (>6.3 mm)	65.0	1.6	76.3

Table 2. Total number of wells sampled for each experimental treatment at each study site.

	Pumping rate ($L \cdot min^{-1}$)					
	Baron Fork		Rock Creek		Saline Creek	
	1.5	4.0	1.5	4.0	1.5	4.0
Temporary wells						
No pores	5	5	0	0	5	5
4-mm pores	5	5	5	5	5	5
6-mm pores	5	5	5	5	5	5
8-mm pores	5	5	5	5	5	5
Permanent wells (6-mm pores)	0	0	4	3	5	5

resist suction of the pump. Well installation and its diameter and shape may modify substrate texture in the well's immediate vicinity, thus affecting sampling success (Tillman et al. 1996). The exact region of the streambed that is sampled also cannot be guaranteed (Palmer and Strayer 1996) because of sediment heterogeneity. Well permanence can also affect sampling results. Hakenkamp and Palmer (1992) demonstrated that organisms colonizing a permanent well differed significantly, in terms of both composition and abundance, from those animals collected from a newly installed well. They also reported that samples taken sequentially from a well cannot be used as replicates and that a 48 h period between sequential samples from the same well did not allow adequate time for recovery by the fauna in the immediate vicinity of the well. Other sources of bias in samples from colonization or permanent wells include the trapping action in the nonperforated segment of such wells and the possible attraction of predators and (or) scavengers (Bretschko and Klemens 1986).

Because of the popularity of the Bou–Rouch method for hyporheic sampling, and because of the variability in its application, the objective of this investigation was to evaluate the effects of various sampling permutations on estimates of basic parameters describing hyporheic invertebrate communities. Four specific permutations were examined in three streams with different sediment composition. Specifically, we investigated how estimates of density and taxon richness were influenced by (*i*) well design, (*ii*) pumping rate, and (*iii*) sample volume. From these results, we develop sampling recommendations that will improve the reliability of data collected by this popular method.

Methods

Study sites

Samples were collected in June and July 1998 from three Oklahoma (U.S.A.) streams of different size and substrate type. Rock Creek is a spring-fed stream with fine to coarse sand bed sediments. Baron Fork is a larger stream and has bed sediments consisting of poorly sorted gravels and smaller cobbles. Sediments in Sager Creek are dominated by small gravel. Physical data describing these streams are presented in Table 1. Information on the hydrology and water chemistry of these streams is presented in Hunt (1999).

Effects of well design and pumping rate

Wells used in this investigation consisted of 2.5-cm (internal diameter) PVC pipe. The tubing was driven into the stream's substratum by inserting a steel T-shaped rod into the well and driving the rod and well to the desired depth. The T-bar was then removed, leaving the well in place.

Five well designs were evaluated. These included four types of temporary wells (wells with no pores and wells with 4-, 6-, or 8-mm pores drilled along the lower 15 cm of the tubing) and one type of permanent well (with 6-mm pores). The perforated areas consisted of four rows evenly spaced along the 15-cm length with eight holes in each row. The perforated wells were installed such that the perforated section was located between 30 and 45 cm below the bottom of the stream. The nonperforated wells were installed such that the open end was located at 37.5 cm below the substrate surface. The intake of the suction hose was positioned at the top of the perforated section of the perforated wells or above the open end of the nonperforated wells. Samples were collected from each temporary well on the date of well installation. Four weeks prior to installation and sampling of the temporary wells, permanent wells were installed in each of the streams. These wells were capped after first simulating sampling by pumping 2.5 L from each well on the day of installation. Samples were then collected from the permanent wells on the same day that the temporary wells were installed and sampled. Ten replicates of each well design were installed in each stream.

To investigate the effect of pumping rate on sampling results, two types of hand pumps were used: a small Nalgene® vacuum pump with a pumping rate of 1.5 $L \cdot min^{-1}$ and a larger diaphragm pump with a 4.0 $L \cdot min^{-1}$ pumping rate. For each set of 10 replicates described above, five replicates were sampled using each of the two pumps. In Rock Creek, the pumping rate was sometimes slowed to prevent sediment from being sucked up with sample water.

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Fig. 1. Comparison of total number of organisms collected from different well designs at two pumping rates at (*a*) Baron Fork, (*b*) Rock Creek, and (*c*) Sager Creek. Values are means + 1 SE.

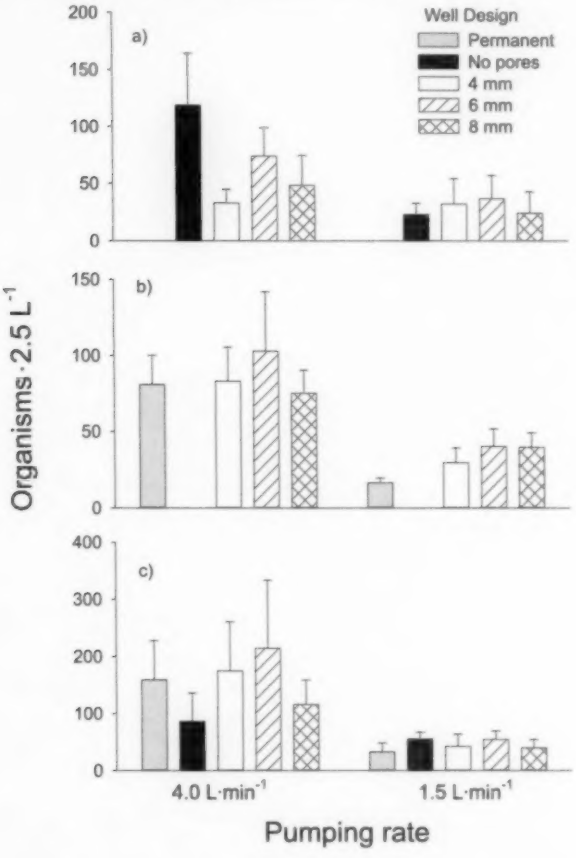
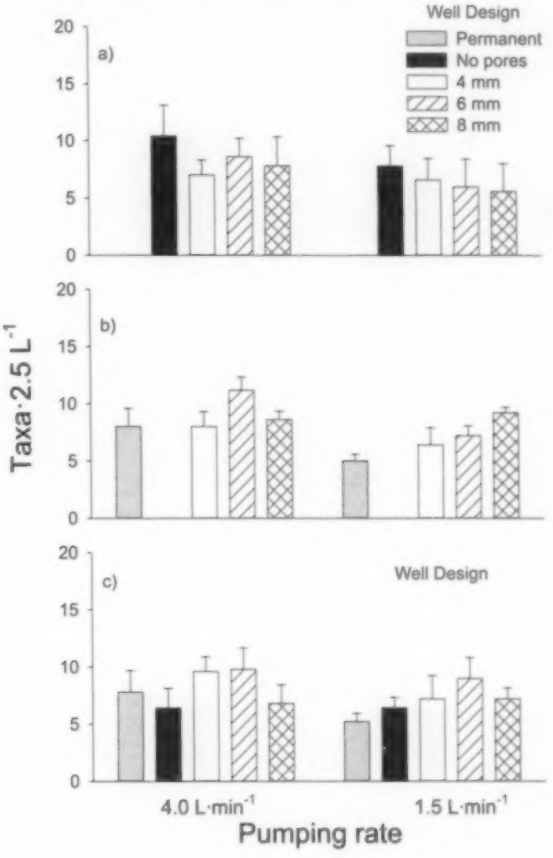


Fig. 2. Comparison of taxon richness (2.5-L samples) collected from varying well designs at two pumping rates at (*a*) Baron Fork, (*b*) Rock Creek, and (*c*) Sager Creek. Values are means + 1 SE.



In each stream, wells were distributed among five blocks, each block containing all combinations of well designs and pumping rates. Thus, each block included two wells of each design, with one well of each design sampled at the slower pumping rate and the second at the faster pumping rate. The five blocks were established in adjacent segments of the stream with similar substrate, depth, and current velocity. Wells within each block were installed randomly and were separated by a minimum of 0.7 m. The numbers of wells of each design and each pumping rate are summarized in Table 2. Nonperforated wells could not be sampled properly in Rock Creek due to the quantity of sand sucked up with the sample water. In addition, all of the permanent wells installed in Baron Fork and three of those installed in Rock Creek were washed out by high storm waters prior to sampling.

Effects of sample volume

To investigate the influence that sample volume has on density estimates, a 2.5-L hyporheic sample was pumped from each well in sequential 0.5-L aliquots. Each 0.5-L aliquot was concentrated with a sieve, placed in a separate labeled jar, and preserved in 5% formalin. Samples collected from Baron Fork and Rock Creek were concentrated with a 63-μm-mesh sieve. Due to frequent high turbidity of the Sager Creek hyporheic waters, samples from this stream were concentrated with a 120-μm-mesh sieve. The larger mesh size used in Sager Creek and the lower pumping rate in Rock Creek did not affect the conclusions, since each stream is treated separately in the analyses. Organisms were sorted to recognizable taxonomic units using a dissecting microscope aided by the addition of rose bengal stain. Although all samples were collected in sequential 0.5-L aliquots, only the data from temporary wells were included in the statistical comparisons of the effects of volume due to the small numbers of permanent wells actually sampled.

Statistical analyses

Differences in invertebrate density and taxon richness based on the five well designs (permanent wells with 6-mm pores and temporary wells with no pores and with 4-, 6-, and 8-mm pores) and two pumping rates within each stream were assessed using a two-way analysis of variance (ANOVA) with repeated measures (blocks). Differences between density (number of individuals per litre) and accumulated taxon richness based on incremental increases in sample volume size, i.e., 0.5, 1.0, 1.5, 2.0, and 2.5 L, were tested for significance using a one-way ANOVA with repeated measures. All tests were performed on $\log_{10}(x + 1)$-transformed data. The analysis did not include the permanent wells in Rock Creek because of the three missing data points. However, data from the seven remaining permanent wells are shown in Figs. 1 and 2. When significance was indicated ($p < 0.05$), multiple comparisons were made using the Student–Newman–Keuls (SNK) procedure.

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Table 3. Composition of the hyporheic fauna at the three study streams.

	Baron Fork			Rock Creek			Sager Creek		
Taxon	%	Mean	SE	%	Mean	SE	%	Mean	SE
Total	100	48.8	9.27	100	62.6	7.73	100	96.0	18.49
Crustacea	57.1	27.8	5.17	45.6	28.5	5.10	73.9	70.5	11.58
Copepoda	28.4	13.8	2.73	42.1	26.4	4.81	44.8	42.6	7.14
Ostracoda	21.0	10.2	2.44	3.3	2.1	0.60	8.3	8.0	1.46
Isopoda	1.8	0.9	0.34	0.0	0.0	0.00	16.3	15.7	4.61
Other Crustacea	5.9	2.9	0.87	0.2	0.1	0.07	4.5	4.2	1.12
Insecta	18.6	9.1	2.20	28.5	17.8	2.28	2.7	2.6	0.55
Acari	3.8	1.9	0.53	3.7	2.3	0.38	1.7	1.7	0.63
Oligochaeta	15.4	7.5	2.04	18.3	11.4	3.23	13.9	13.5	7.74
Nematoda	1.5	0.7	0.30	0.4	0.3	0.08	7.6	7.4	2.56
Other invertebrates	3.7	1.8	0.52	3.5	2.2	0.44	0.3	0.3	0.08

Note: Percentage, mean number of individuals, and ±SE are based on a sample size of 2.5 L. All values are based on averages from all pumping rates and well designs.

Table 4. Summary of results of two-way ANOVA with repeated measures to determine the effects of well type and pumping rate on estimates of hyporheic densities as number of individuals per 2.5 L.

Stream	Source of variance	df	*F*	*p*
Baron Fork	Block	4		
	Pumping rate	1	1.70	0.26
	Well type	3	0.65	0.60
	Pumping rate × well type	3	0.46	0.71
Rock Creek	Block	4		
	Pumping rate	1	11.89	0.03
	Well type	3	2.04	0.19
	Pumping rate × well type	3	1.41	0.30
Sager Creek	Block	4		
	Pumping rate	1	22.04	0.009
	Well type	3	0.42	0.79
	Pumping rate × well type	3	1.84	0.17

Note: See Table 2 for specific treatments at each stream.

Table 5. Summary of results of two-way ANOVA with repeated measures to determine the effects of well type and pumping rate on estimates of hyporheic taxonomic richness as number of major groups per 2.5 L.

Stream	Source of variance	df	*F*	*p*
Baron Fork	Block	4		
	Pumping rate	1	0.94	0.39
	Well type	3	0.71	0.57
	Pumping rate × well type	3	0.43	0.73
Rock Creek	Block	4		
	Pumping rate	1	31.09	0.005
	Well type	3	2.44	0.15
	Pumping rate × well type	3	1.55	0.27
Sager Creek	Block	4		
	Pumping rate	1	0.78	0.43
	Well type	3	1.47	0.26
	Pumping rate × well type	3	0.90	0.49

Note: See Table 2 for specific treatments at each stream.

Results

Taxonomic composition

The three streams represented three distinctly different hyporheic invertebrate communities (Table 3). Average densities (based on all samples) ranged from 49 to 96 organisms per 2.5-L sample in Baron Fork and Sager Creek, respectively. Four groups, i.e., Copepoda, Ostracoda, Insecta (predominantly Chironomidae), and Oligochaeta, together composed 75% of all organisms in Baron Fork. The three most abundant groups in Rock Creek (83%) were Copepoda, Insecta, and Oligochaeta. Other crustaceans were rarer than in the other two streams. Density was greatest in Sager Creek, even though the larger mesh size was used in sample collection. Crustacea was the dominant taxonomic group (74%); copepods and isopods together composed 60% of the organisms collected in this stream. Oligochaeta, Ostracoda, and Nematoda were also more abundant in Sager Creek than in either Rock Creek or Baron Fork.

Effects of well design and pumping rate

Density estimates (number of organisms per 2.5-L sample) were significantly different between the two pumping rates across all well types at Rock and Sager Creeks (Table 4; Fig. 1). While average values were higher for the fast pumping treatment at Baron Fork, this difference was not significant. There was no effect of well design at any of the three streams.

Trends in taxon richness were less pronounced. Pumping rate had a significant effect on richness only at Rock Creek (Table 5; Fig. 2), and well type again had no significant effect.

Effects of sample volume

On average, 50–55% of all organisms collected in a 2.5-L sample were collected in the first 0.5-L aliquot at each of the three streams, and the percentage of total individuals collected decreased with consecutive 0.5-L aliquots. Consequently, density estimates of total individuals and of major taxonomic groups based on each of the five sample volumes decreased with increasing volume (Fig. 3). Density estimates based on different sample volumes were significantly different in all three streams (one-way ANOVA with repeated measures: $F = 58.1$, 258.2, and 155.7 for Baron Fork, Rock Creek, and Sager Creek, respectively, df = 4, $p < 0.001$ for all streams). Indeed, density estimates decreased signifi-

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Fig. 3. Comparison of mean density (+1 SE) of the hyporheic fauna in Baron Fork, Rock Creek, and Sager Creek calculated from five consecutive sample volumes. Breaks in the horizontal line above bars indicate where statistically significant differences occur between density estimates.

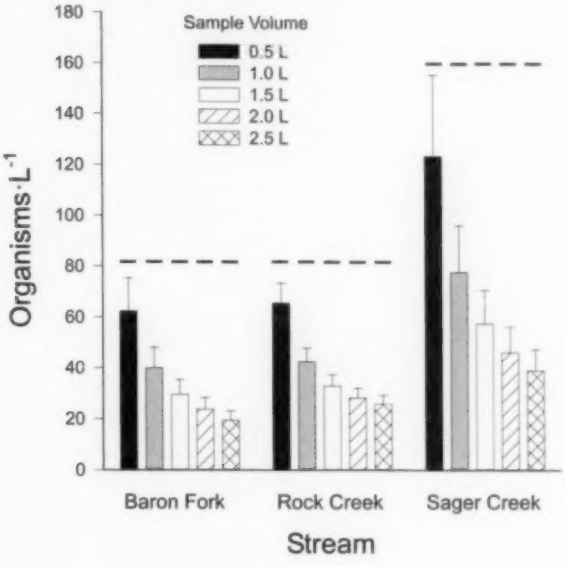
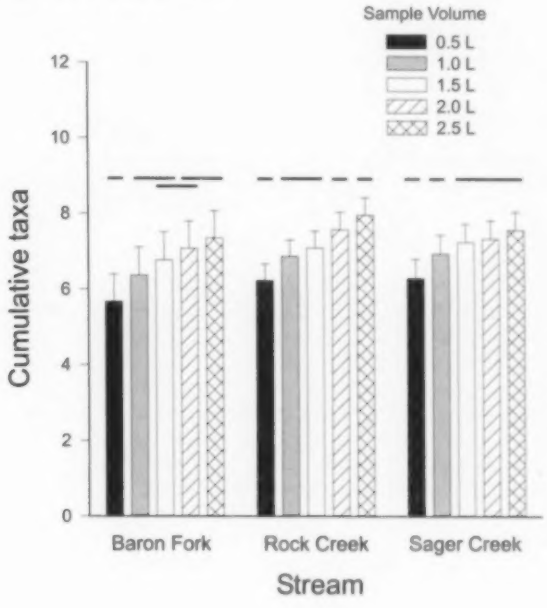


Fig. 4. Comparison of mean taxon richness (+1 SE) of the hyporheic fauna in Baron Fork, Rock Creek, and Sager Creek estimated from five sample volumes. Breaks in the horizontal line above bars indicate where statistically significant differences occur between richness estimates. Means are based on the total number of taxa per sample.



cantly ($p < 0.05$) with each increase in volume, i.e., 0.5 L > 1.0 L > 1.5 L > 2.0 L > 2.5 L (SNK multiple comparisons).

As with density, estimates of taxon richness based on different sample volumes differed significantly in all three streams (one-way ANOVA with repeated measures: F = 23.4, 36.9, and 22.0 for Baron Fork, Rock Creek, and Sager Creek, respectively, df = 4, $p < 0.001$ in all cases) (Fig. 4). SNK multiple comparisons indicated that the larger sample volumes provided significantly greater estimates of total taxon richness ($p < 0.05$).

Discussion

The results of our investigation underscore the importance of maintaining consistent methodologies when sampling hyporheic fauna using the Bou–Rouch method. We found that sample size and pumping rate both significantly affected density estimates in a range of different sediment types. These results provide an interesting contrast with the diversity of (largely untested) recommendations regarding optimal sampling strategies using the Bou–Rouch method that exists in the literature. For example, Danielopol (1976) suggested that the first 10-L sample from a well is the most productive and perhaps provides the best density estimate for organisms living in close proximity to sediments, including nematodes, oligochaetes, and harpacticoids. Conversely, Pospisil (1992) emphasized the importance of the origin of hyporheic water and the selective influence that sediment size can have on the water's origin. He suggested that limiting the sample volume guarantees that water is taken from a well-defined zone. Other investigators recommend that the first 0.2–0.5 L of water from a well be discarded to avoid the risk of contamination with surface water and its biota (Boulton et al. 1992) or because permanent wells may serve as traps selectively accumulating specific taxonomic groups such that the first few litres may not be representative of the hyporheic fauna (Pospisil 1992).

The enormous effect of sample volume on density estimates described here suggest that the first 0.5 L removed from a temporary well may be the best quantitatively representative sample that can be obtained by the Bou–Rouch method. We could not address the concern that the nature of the sample from a permanent well changes with continued pumping and thus that the first aliquot of water from permanent wells should be discarded (Pospisil 1992), as sample volume effects were evaluated only for newly installed (temporary) wells. Yet, well permanence did not significantly affect estimates of density or richness for 2.5-L samples. While collection of large volumes of water increase the likelihood of collecting rare taxa, it would appear that the net effect of removing progressively more water from the hyporheic zone is to underestimate population densities and volume-specific estimates of richness. Sample volume is clearly a critical factor that must be given greater emphasis in quantitative studies using the Bou–Rouch method.

We are unaware of any studies that have considered the influence that well design may have on sampling of the hyporheic fauna. Typically, 5-mm (e.g., Danielopol 1976) or 6-mm perforations (e.g., Pennak and Ward 1986) have been used, although use of open-ended, unperforated wells is also widespread (e.g., Boulton and Stanley 1995). We expected that increasing the surface area of the bed sediments exposed to the influence of the pump's suction would reduce the

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screening action of the sediments, as described by Williams (1984), and thus would affect estimates of both density and richness, at least for smaller sample volumes. However, in this study the differences in total number of organisms from wells of varying design, including permanent colonization wells, were not significantly different, regardless of sediment composition.

Pumping rate strongly influenced estimates of hyporheic density at two of the three sites and richness at one of the three sites. Organisms adhering less tightly to the substrate, e.g., cyclopoids, ostracods, isopods, and amphipods, may be more readily captured by pumping (Danielopol 1976). Thus, stronger pumping rate may capture those taxa that have some ability to resist mild vacuum pressure produced by smaller hand-held pumps. In streams with smaller sediments, such as Rock Creek, pumping rate often has to be adjusted to minimize the quantity of sediments sucked into the sample. The optimal pumping rate obviously will vary with bed sediment type; however, perhaps the optimum rate is the maximum rate that can be used.

In conclusion, recommendations for sampling are dependent on the intentions of the work to be undertaken. If the primary objective is to obtain reliable estimates of density, we suggest the use of high pumping rates to collect small volumes of water, regardless of well design. If the goal is to maximize the total number of taxa collected, then traditional approaches of extracting large sample volumes (again at a high pumping rate) are appropriate. In any case, future studies using the Bou–Rouch method should report the pumping rate. Despite these recommendations, substantial work remains in assessing hyporheic sampling protocols. The large effects of sample volume and pumping rates demonstrated here indicate that attempts to draw comparisons among different studies would be speculative. Consequently, such limitations similarly restrict large-scale syntheses of the ecology of hyporheic invertebrates. Clearly, grappling with variations among different sampling techniques is a necessary hurdle to overcome toward the goal of understanding these poorly studied organisms.

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An Atlantic herring (*Clupea harengus*) size selection model for experimental gill nets used in the Sound (ICES Subdivision 23)

Søren Poulsen, J. Rasmus Nielsen, René Holst, and Karl-Johan Stæhr

Abstract: Size selection investigations were performed in the Sound with experimental, multipanel gill nets equipped with a broad range of mesh sizes targeting Atlantic herring (*Clupea harengus*). Each of the 20 experimental fishery surveys covered the central Sound from Helsingør–Helsingborg (north) to Drogden–Klagshamn (south) during the autumn, winter, and spring periods from 1993 to 1998. There was high variability in catch sizes both between and within surveys. The overall best fits, in terms of minimum total deviance across all sets, were achieved using a unimodal normal scale model. Almost all surveys showed high between-set variation, which was accounted for when estimating the mean selectivity curves using a random effects model. The selectivity curves fitted to individual sets were compared with those fitted to the combined data sets. It was demonstrated that analysing pooled data sets resulted in overdispersion and bias in the selection parameters. Irregular time series of the estimates of the selectivity parameters in the investigation period indicated negative autocorrelation with a seasonal pattern.

Résumé : Nous avons mené dans le Øresund des études sur la sélection par taille du hareng atlantique (*Clupea harengus*) à l'aide de filets maillants expérimentaux constitués d'un certain nombre de panneaux présentant une série de maillages très différents. Chacune des 20 pêches expérimentales a couvert le centre du détroit, de Helsingør–Helsingborg (au nord) à Drogden–Klagshamn (au sud), pendant l'automne, l'hiver et le printemps, de 1993 à 1998. Nous avons noté une forte variabilité de la taille des captures dans chaque pêche et entre les pêches. Nous avons établi les meilleurs ajustements globaux, en termes d'écart total minimum pour toutes les séries, à l'aide d'un modèle unimodal à échelle normale. Pour presque toutes les pêches, on observait une forte variation d'un trait à l'autre, et nous en avons tenu compte pour calculer les courbes de la sélectivité moyenne à l'aide d'un modèle à effet aléatoire. Nous avons comparé les courbes de sélectivité ajustées à chaque trait aux courbes ajustées aux séries combinées de données. Il est apparu que l'analyse des séries de données regroupées provoquait une surdispersion et du biais dans les paramètres de sélection. L'irrégularité chronologique dans les estimations des paramètres de sélectivité pendant la période d'étude indiquait la présence d'une autocorrélation négative avec patron saisonnier.

[Traduit par la Rédaction]

Introduction

Results on tagging experiments and information from fishery indicate that the Sound (ICES Subdivision 23) is a major overwintering area for the spring-spawning Rügen (western Baltic) Atlantic herring (*Clupea harengus*) stock and that the main component of herring in the Sound is RHS (Biester 1979; Jönsson and Biester 1981; Otterlind 1987). The Rügen herring stock is a significant resource for the Danish, German, Norwegian, and Swedish commercial fishery in the Baltic Sea, Kattegat–Skagerak, and, to a lesser extent, the North Sea, respectively (ICES 1999). The present investigation is a part of a project giving the first detailed, quantitative estimates of occurrence and abundance of the overwintering Rügen herring in the Sound and the passage of migrating herring through the Sound repeatedly on seasonal basis over an extended time period of 6 years. During the period September 1993 – May 1998, 27 hydroacoustic monitoring surveys were carried out in the central Sound covering all seasons of the year. Parallel to the hydroacoustic data sampling, biological data sampling with experimental (scientific), multipanel gill nets equipped with a broad spectrum of mesh sizes was performed. These gill nets are highly selective. In order to allocate representative size distributions of herring from the biological data to the acoustic data, it is necessary to estimate the size selection of the gill nets. The intention of the present study is to evaluate the selectivity of the multipanel monofilament gill nets for herring in order to measure the abundance (biomass) as well as to describe the horizontal and vertical distribution and density patterns of herring in the Sound. When estimating gear selectivity over a wide range of years and seasons, it is relevant to test for changes in the gillnet selectivity over that time span.

Gill nets are considered highly selective fishing gears, and a gill net of a given mesh size will tend to be most efficient

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S. Poulsen,[1,2] **J.R. Nielsen,**[1] **and K.-J. Stæhr.**[1] Danish Institute for Fisheries Research (DIFRES), Department of Fish Biology, North Sea Centre, P.O. Box 101, DK-9850 Hirtshals, Denmark.
R. Holst.[1] ConStat, North Sea Centre, P.O. Box 104, DK-9850 Hirtshals, Denmark.

[1]Authorship equal. Correspondence to all authors.

[2]Present address: Novo Nordisk IT A/S, Desktop Training, Building 89US.39-BA, DK-2880 Bagsvaerd, Denmark.

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at catching fish of a certain size range. Generally, few fish are caught whose lengths differ from the optimum by more than 20% (Baranov 1948). The very selective exploitation pattern of gill nets has, however, created difficulties in the interpretation of gillnet catch statistics in herring stock assessment studies (O'Boyle and Cleary 1981; Ahrens 1985; Hansson and Rudstam 1995). Numerous studies of gillnet selectivity have been carried out for a wide variety of species (Hamley 1975; Holst and Moth-Poulsen 1995). The basis of most of these studies was the classical work of Baranov (1948).

Gillnet selectivity studies are frequently used to estimate the abundance and size structure of fish populations, particularly where trawls cannot be used (e.g., Hansson and Rudstam 1995). Due to lack of nonselective gears, it is necessary to use several mesh sizes simultaneously in order to obtain estimates of the selectivity. Selectivity is then estimated by imposing assumptions on the selectivity at length-classes across mesh sizes. In the majority of the gillnet selectivity studies, the selectivity curve takes the form of a unimodal curve (Hamley 1975). Winters and Wheeler (1990) found, however, indications of bimodal selectivity curves for herring. Similarly, Hovgård (1996) found that Atlantic cod (*Gadus morhua*) from Greenland gillnet surveys displayed a binormal selectivity curve. He also attributed the two components to different capture processes (gilling and maxillae enmeshing). Millar (1992) demonstrated the difficulties in determining the shape of the selectivity curve by comparing goodness-of-fits for a variety of different curves.

Numerous approaches for estimating gillnet selectivity have been suggested over time (Holt 1963; Hovgård 1996; Millar and Holst 1997). Only the latter provides a rigorous statistical model for the selection process and is based on the general SELECT method (Millar 1992). Kirkwood and Walker (1986) and Wulff (1986) used techniques that are special cases of the SELECT method. Holst and Moth-Poulsen (1995) compared the performance of six different methods with a number of different data sets and discussed the strengths and weaknesses in each model. Holst and Moth-Poulsen (1995) stated that most of the concerned methods suffered from lack of estimates with known properties. The well-known and attractive asymptotic properties of the maximum likelihood estimators make maximum likelihood based methods preferable. In the present study, the fitting of selectivity curves to the sea trials catch data was done using the SELECT model (Millar 1992; Millar and Holst 1997). This model provides maximum likelihood estimates of the selectivity parameters.

Materials and methods

The study area covers the central Sound (ICES Subdivision 23) from Helsingør (56°02′N, 12°37′E) – Helsingborg (56°03′N, 12°41′E) in the north to Drogden (55°31′N, 12°43′E) – Klagshamn (55°32′N, 12°54′E) in the south (Fig. 1).

Sampling was carried out on the RV *Havkatten* (DIFRES) during 20 individual surveys from October 1993 to April 1998 with seven, seven, and six cruises in the spring, summer, and winter, respectively. The sampling was carried out as a parallel approach to hydroacoustic monitoring (echo integration) in order to study aggregations of the spring-spawning Rügen Atlantic herring stock at its a main overwintering ground (Nielsen et al. 1999).

Table 1. Characteristics of the gill nets used in the selectivity study.

Mesh size (mm)	Height (m)	Length (m)	Area (m^2)
19	5.7	30	171.0
21	5.7	30	171.0
26	5.0	39	195.0
27	5.0	39	195.0
28	5.4	40	216.0
29	5.0	40	200.0
34	5.1	34	173.4

All nets were made of monofilament polyamid mounted with a nominal twine thickness of 5–6 mm and a hanging ratio of 0.5. The multipanel gill net was constructed by combining four to seven nets with mesh sizes of 18.5, 21, 26, 27, 28, 29, and 34 mm (inside stretched), respectively. The nets were designed to match a mounted size of 34–40 m in length and 5.0–5.7 m in depth. The exact measurements of all net types used are presented in Table 1. Headrope floatation was achieved using torpedo floats mounted on a 5.0-mm polypropylene line, which produced buoyancy of 180–200 $g \cdot m^{-1}$. The footrope was made of lead line with a weight of 500 $g \cdot m^{-1}$, thus leaving an open space of 1 m where the individual nets were tied together. This design was chosen to reduce the effect of larger fish being led by a small mesh sized net into a neighboring net of a more suitable size.

Individual sets for sampling of herring were made in each geographical stratum on both the Danish and Swedish sides of the Sound. Depending on time, weather, and water currents, the fishing stations were stratified to cover both demersal and pelagic water layers. Fishing trials were mainly carried out during the nighttime in synchrony with acoustic integration. The total number of sets was 206, of which 160 were classified as being valid for the present gear selectivity analyses. Sets were deemed invalid if estimation of a selectivity curve did not succeed. This was typically due to small catches. A few sets, where selectivity estimates had been obtained, were discarded if the estimates appeared physically unrealistic. The number of valid sets within each survey ranged from 3 to 12 (Table 2). The gillnet fishery and the choice of gear were designed in order to cover as large a geographical part and as many depths strata as possible in the Sound area. Furthermore, the gillnet fishery was directed towards the 2+ age group of herring (the spring-spawning Rügen herring stock component in the Sound).

Catches were sorted by fish species for each mesh size separately. For each mesh size, the total lengths of all fish were measured to the nearest semicentimetre below for clupeoids (herring and sprat (*Sprattus sprattus*)) and to the nearest centimetre below for all other species, and the total catch weight (grams) was recorded for each species. Fish falling out during handling of the nets were excluded from the analysis. Bycatch of species other than herring was less than 2% (in numbers) of the total gillnet catch and consisted mainly of cod, whiting (*Merlangius merlangus*), and garfish (*Belone belone*). The analysis was based on a total of 27 765 herring specimens caught.

Estimation procedures

Let $N_{l,j}$ denote a random variable representing the number of length-class l fish caught in the jth mesh size m_j. The corresponding observed value of $N_{l,j}$ is denoted by $n_{l,j}$. Following the SELECT approach (Millar 1992), it is natural to model these as Poisson random variables with rates given by

$$E(N_{l,j}) = \lambda_l p_j r_j(l)$$

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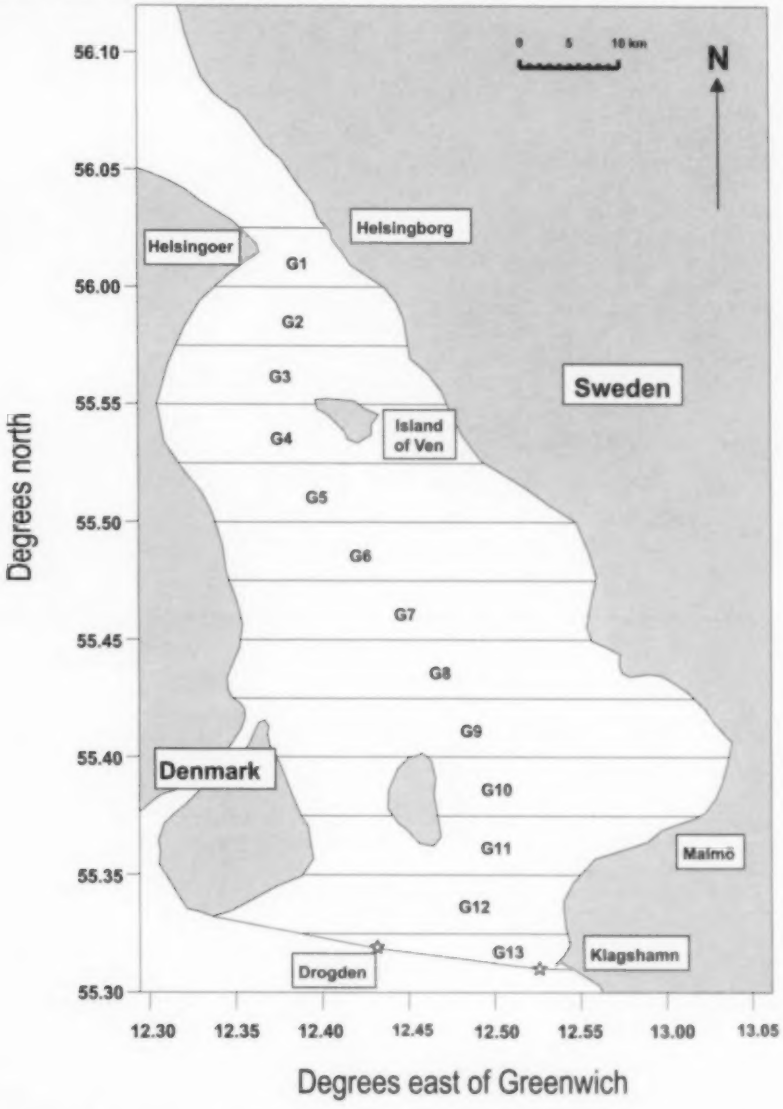
Fig. 1. Survey area covering the central Sound (ICES Subdivision 23) from Helsingør–Helsingborg in the north to Drogden–Klagshamn in the south. The survey area is divided by geographical strata G1–G13, which all are approximately 2.5 nautical miles wide.



where λ_l is the expected number of length-class l fish encountering the combined set of nets and p_j is the relative fishing intensity of the jth mesh. If we assume the same fishing power (i.e., maximum height of selection curve) of all mesh sizes, the relative fishing intensities will just be the relative fishing efforts (Millar and Fryer 1999). The $r_j(l)$ is the relative retention probability of length-class l fish in mesh size m_j. The $r_j(l)$s are conveniently chosen to be members of a common parametric family $r_j(l;\theta)$. The parameter vector θ is then estimated by maximizing the likelihood function. A common choice for $r_j(l;\theta)$ is the normal scale.

$$r_j(l; k_1, k_2) = \exp\left(\frac{(l - k_1 m_j)^2}{2(k_2 m_j)^2}\right)$$

The parameters k_1 and k_2 describe the location and spread respectively of the selection curve per unit mesh size. Millar and Holst (1997) demonstrated how the general model reduced to a log linear model for a number of different unimodal selectivity curves including the normal scale.

Experience indicates, however, that these provide very poor fits for most fish species. Furthermore, a combination of two normal scales seems to be a good candidate for a general alternative to the more simple selectivity curves.

Estimation of the parameters is achieved by maximizing the log likelihood function, which, except for a constant, is given by

$$L(\theta) = \sum_{j,l} n_{l,j} \log(\phi_j(l;\theta))$$

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Table 2. Summary of Atlantic herring survey activities and sampling.

Survey No.	Sampling period	No. sets used: Survey	No. sets used: Analysis	No. mesh sizes used	Catch
93s02	25–29 Oct. 1993	15	11	4	1282
93s03	29 Nov. – 3 Dec. 1993	5	5	6	140
94s04	10–14 Jan. 1994	10	9	6	1547
94s06	15–17 Mar. 1994	9	7	5	498
94s07	11–14 Apr. 1994	12	9	6	796
94s10	18–27 Oct. 1994	17	12	6	2456
94s11	21–27 Nov. 1994	15	8	7	1452
94s12	12–15 Dec. 1994	13	11	7	2279
95s01	9–15 Jan. 1995	11	10	7	2513
95s02	6–10 Feb. 1995	11	10	7	2689
95s03	27 Feb. – 3 Mar. 1995	9	8	7	268
95s04	28 Mar. – 1 Apr. 1995	15	11	7	437
95s09	10–15 Oct. 1995	3	3	7	2231
96s01	18–21 Mar. 1996	12	11	7	1664
96s10	30 Sept. – 6 Oct. 1996	3	3	7	2257
96s11	11–17 Nov. 1996	3	3	7	1507
97s03	4–9 Feb. 1997	12	8	7	388
97s04	7–10 Mar. 1997	10	8	7	848
97s11	11–15 Nov. 1997	3	3	7	2195
98s02	30 Mar. – 7 Apr. 1998	18	11	7	318
Total		206	160		27 765

where

$$\phi_j(l;\theta) = \frac{p_j r_j(l;\theta)}{\sum_i p_i r_i(l;\theta)}$$

is the expected proportion of length-class l fish caught in the jth net.

In the case where the catches are subsampled, this is accounted for by the sampling fractions q_j (either by weight or by numbers) as an extra term in the model. The model thus becomes

$$E(N^*_{l,j}) = \lambda_l p_j q_j r_j(l)$$

where $N^*_{l,j}$ is the random variable measuring the subsampled catch of length-class l fish in the the jth mesh size. Effectively, this is simply an adjustment of the effort parameter. Alternatively, the observations in the subsampled mesh sizes could be divided by the sampling fractions or the catches in the other mesh sizes could be reduced accordingly. In both cases, the selectivity parameters are likely to be estimated at about the same level, but their variances will be under- or over-estimated, respectively.

Within a given survey, a number of sets are conducted. In the following, we omit reference to the survey. Provided that sufficient fish are caught and not clustered in too few length-classes or too few mesh sizes, the ith set will yield an estimate $\hat{\theta}_i$ of the selectivity parameters θ_i for that set. Due to the asymptotic properties of the maximum likelihood estimator, $\hat{\theta}_i$ can be assumed normal with mean θ_i (Lehmann 1983):

$$\hat{\theta}_i \sim N(\theta_i, R_i).$$

It is generally accepted that selectivity varies between sets, even though all controllable conditions have been kept unchanged. Changes in variables that cannot be controlled or observed or are of no direct interest are classified as random variation. During a survey, the interest is on the mean selectivity curve and the effects of any controlled variables that are accounted for by the experimental design. If the selectivity measured within the survey is represented by a mean selectivity curve, given by θ, the selectivity parameters from the individual sets can be considered samples from a normal distribution (Fryer 1991):

$$\theta_i \sim N(\theta, D).$$

Combining the above leads to a model for a mean selectivity with the between-set variation extracted in a separate variance component:

$$\hat{\theta}_i \sim N(\theta, R_i + D).$$

where R_i measures the within-set variation due to the multinomial error and D measures the random between-set variation described above.

Maximum likelihood or residual maximum likelihood estimates are obtained by use of the E-M algorithm (Dempster et al. 1977). The latter is preferred when relatively few sets are available because the degrees of freedom lost due to estimating the fixed effects in the maximum likelihood approach result in the variance estimators being biased downwards (Laird and Ware 1982).

The model that we used to fit the mean selectivity curve was originally developed by Laird and Ware (1982) as a framework for analysing longitudinal data with between-subject variability. Fryer (1991) described how it could be used for handling between-haul variability for experiments with towed gears. It is, however, equally applicable to gill nets and other static gears, provided the same assumptions are met, and was first published in this context by Madsen et al. (1999) Also see Wileman et al. (1998) for a study that includes effects of controlled changes in gear parameters. In the original Laird–Ware model, the within-subject variances were considered unknown and a part of the parameters to be estimated. When this model is used in the fishing gear selectivity context, the observations are not observations in the traditional sense, but they emerge as estimates of parameters along with estimates of their covariance matrices from an initial analysis. The R_i covariance matrices are therefore considered known, and we substitute them with

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Table 3. Summary of the selectivity estimates and their goodness of fit.

Survey No.	Sampling period	No. sets used (analysis)	Estimate	k_1	k_2	Goodness of fit (p value)
93s02	Oct. 1993	11	REML	2.151	0.1949	0.733
			Maximum	2.311	0.2446	0.978
			Minimum	1.958	0.0951	0.018
			Pooled	2.178	0.2242	0.000
93s03	Nov.–Dec. 1993	5	REML	2.190	0.2183	0.643
			Maximum	2.260	0.2912	0.995
			Minimum	2.060	0.1706	0.136
			Pooled	2.193	0.2404	0.185
94s04	Jan. 1994	9	REML	2.111	0.1794	0.606
			Maximum	2.185	0.2467	0.935
			Minimum	1.998	0.1047	0.000
			Pooled	2.124	0.2469	0.000
94s06	Mar. 1994	7	REML	2.150	0.1876	0.705
			Maximum	2.311	0.2763	0.998
			Minimum	1.939	0.1018	0.238
			Pooled	2.225	0.2339	0.000
94s07	Apr. 1994	9	REML	2.215	0.1747	0.802
			Maximum	2.341	0.2123	1.000
			Minimum	2.090	0.1190	0.112
			Pooled	2.231	0.1877	0.000
94s10	Oct. 1994	12	REML	2.127	0.2112	0.471
			Maximum	2.310	0.5943	1.000
			Minimum	0.568	0.1386	0.000
			Pooled	2.112	0.2328	0.000
94s11	Nov. 1994	8	REML	2.147	0.1911	0.467
			Maximum	2.293	0.2219	1.000
			Minimum	2.040	0.1485	0.000
			Pooled	2.137	0.2040	0.000
94s12	Dec. 1994	11	REML	2.094	0.1923	0.508
			Maximum	2.228	0.2754	1.000
			Minimum	1.900	0.0806	0.000
			Pooled	2.105	0.2263	0.000
95s01	Jan. 1995	10	REML	2.116	0.2111	0.202
			Maximum	2.257	0.6095	0.943
			Minimum	1.913	0.1296	0.000
			Pooled	2.131	0.2666	0.000
95s02	Feb. 1995	10	REML	2.161	0.2071	0.421
			Maximum	2.286	0.2795	1.000
			Minimum	1.956	0.1668	0.000
			Pooled	2.208	0.2040	0.000
95s03	Feb.–Mar. 1995	8	REML	2.094	0.1524	0.904
			Maximum	2.369	0.2422	1.000
			Minimum	1.943	0.0891	0.347
			Pooled	2.094	0.1524	0.890
95s04	Mar.–Apr. 1995	11	REML	2.151	0.1557	0.849
			Maximum	2.363	0.2245	1.000
			Minimum	1.990	0.0746	0.003
			Pooled	2.202	0.2022	0.053
95s09	Oct. 1995	3	REML	2.179	0.2188	0.024
			Maximum	2.207	0.2298	0.072
			Minimum	2.135	0.2003	0.000
			Pooled	2.176	0.2197	0.000
96s01	Mar. 1996	11	REML	2.148	0.1649	0.775
			Maximum	2.238	0.2416	1.000
			Minimum	2.006	0.1164	0.000
			Pooled	2.179	0.2007	0.000

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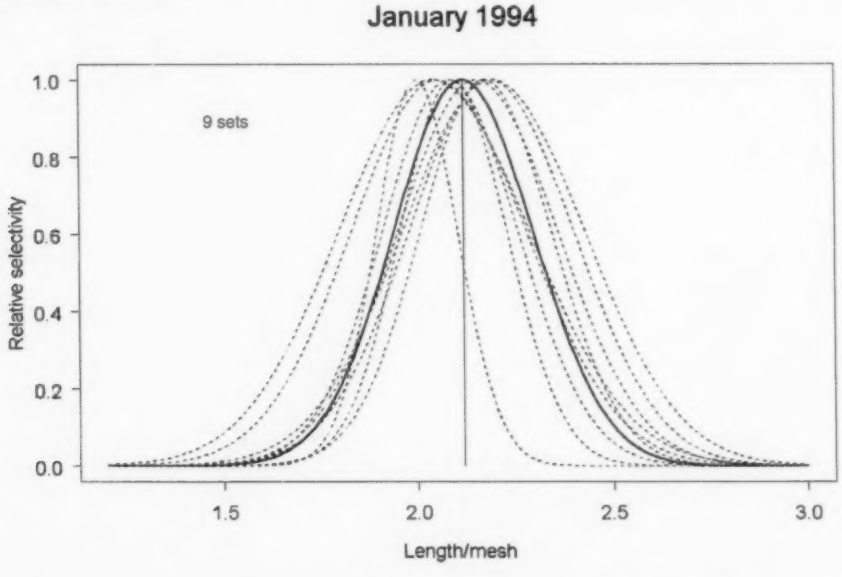
Table 3 (*concluded*).

Survey No.	Sampling period	No. sets used (analysis)	Estimate	k_1	k_2	Goodness of fit (p value)
96s10	Oct. 1996	3	REML	2.132	0.2228	0.331
			Maximum	2.186	0.2305	0.994
			Minimum	2.062	0.2119	0.000
			Pooled	2.158	0.2268	0.000
96s11	Nov. 1996	3	REML	2.139	0.2035	0.332
			Maximum	2.149	0.2328	0.983
			Minimum	2.104	0.1701	0.000
			Pooled	2.146	0.2097	0.000
97s03	Feb. 1997	8	REML	2.150	0.1758	0.737
			Maximum	2.238	0.3566	0.997
			Minimum	1.987	0.1000	0.000
			Pooled	2.131	0.2056	0.000
97s04	Mar. 1997	8	REML	2.184	0.1784	0.846
			Maximum	2.269	0.2119	1.000
			Minimum	2.057	0.1250	0.196
			Pooled	2.219	0.1987	0.000
97s11	Nov. 1997	3	REML	2.197	0.2046	0.000
			Maximum	2.247	0.2494	0.000
			Minimum	2.135	0.1791	0.000
			Pooled	2.211	0.2246	0.000
98s02	Mar.–Apr. 1998	10	REML	2.217	0.1803	0.982
			Maximum	2.578	0.3212	1.000
			Minimum	2.134	0.0677	0.841
			Pooled	2.243	0.2373	0.666

Note: REML, residual maximum likelihood. The selection factor (k_1) and selection spread (k_2) are calculated for both individual and combined (pooled) data.

Fig. 2. Example of mean (solid line) and individual set selectivity curves (broken lines) for Atlantic herring caught in the January 1994 survey. The mean curve was estimated using 9 individual data sets. Length/mesh is fish length divided by mesh size. The curve has a modal value (selection factor) at k_1.



their estimates $\hat{R}_l$ in the E-M algorithm. Details can be found in Fryer (1991).

For the purpose of estimating the selectivity, the λ_ls are considered nuisance parameters and act only indirectly in the estimation (McCullagh and Nelder 1989). If, however, the experiment is designed for obtaining an index of the stock, λ_l can be estimated by

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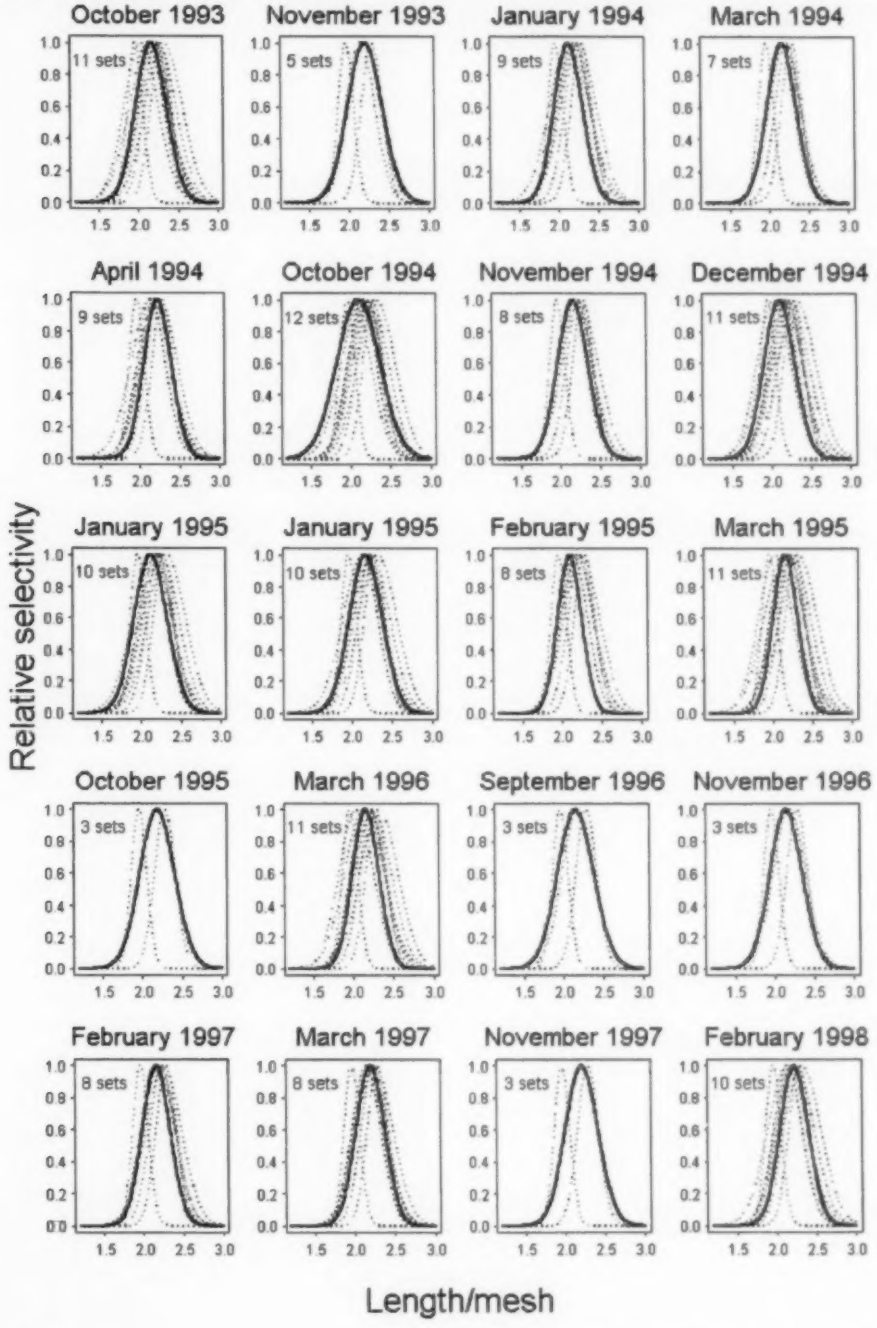
Fig. 3. Residual maximum likelihood mean (solid line) and individual set selectivity curves (dotted lines) for Atlantic herring caught during each survey in the period October 1993 – February 1998. The number of sets used in each survey is shown in each panel.



$$\hat{\lambda}_l = \frac{\sum_j n^*_{l,j}}{\sum_j p_j q_j r_j(l;\hat{\theta})}.$$

Further methodological aspects in relation to the selection model are discussed in Millar and Holst (1997).

Results

A number of different selectivity curves were fitted to the

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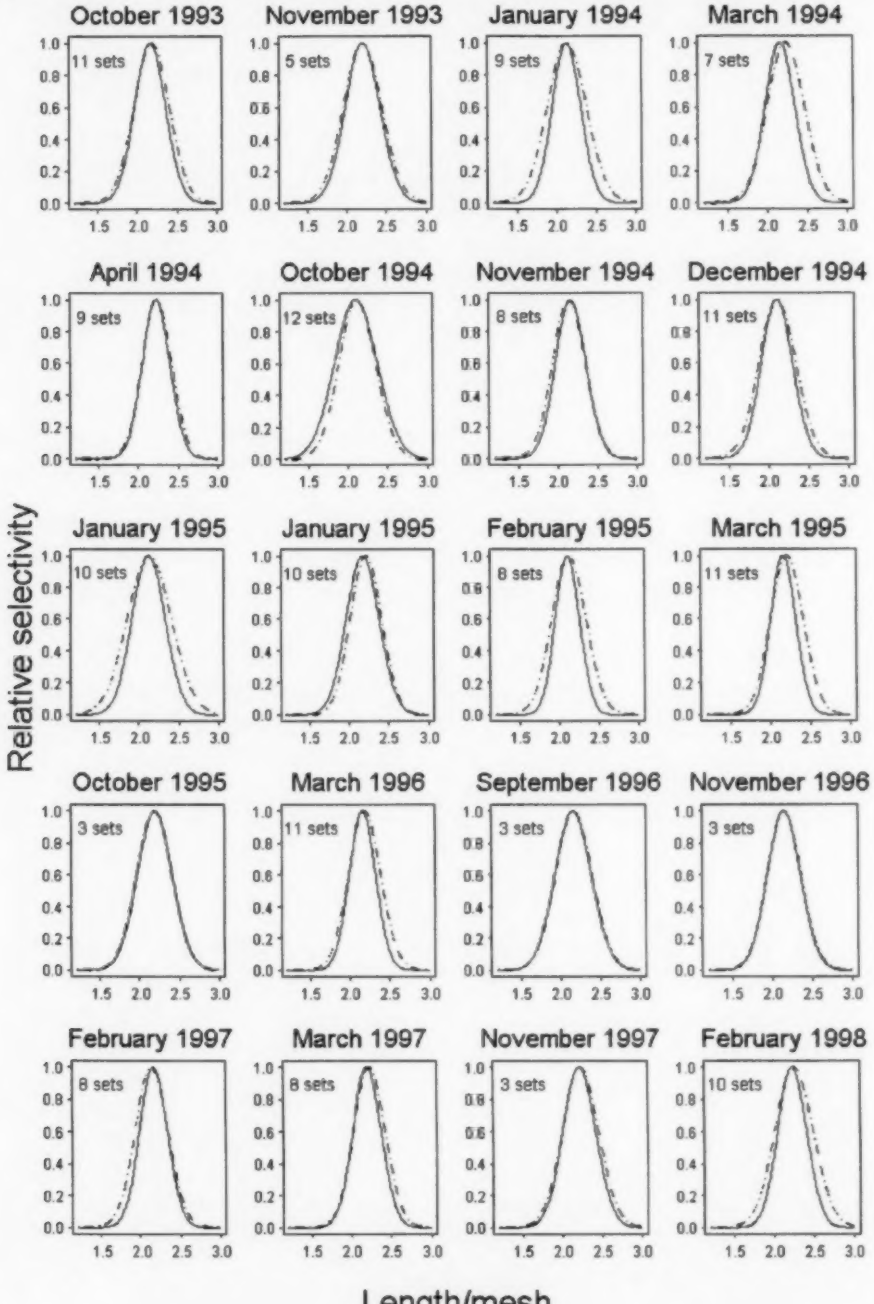
Fig. 4. Mean and individual set selectivity curves for Atlantic herring caught during each survey in the period October 1993 – February 1998. The mean curve estimated using individual data sets (solid line) (number shown in each panel), the mean curve estimated using a combined (pooled) data set (dashed line), and the curve for individual sets (dotted line) are presented.



data. These included the four unimodal curves described by Millar and Holst (1997): normal location, normal scale, gamma, and lognormal. All except the normal location observes the principle of geometrical similarity (Baranov 1948), i.e., the spread and modal length of the selectivity curves are proportional to the mesh sizes. Furthermore, a bimodal curve (a mixture of two normal scales) was also fitted to each set. Selectivity curves were fitted to individual sets

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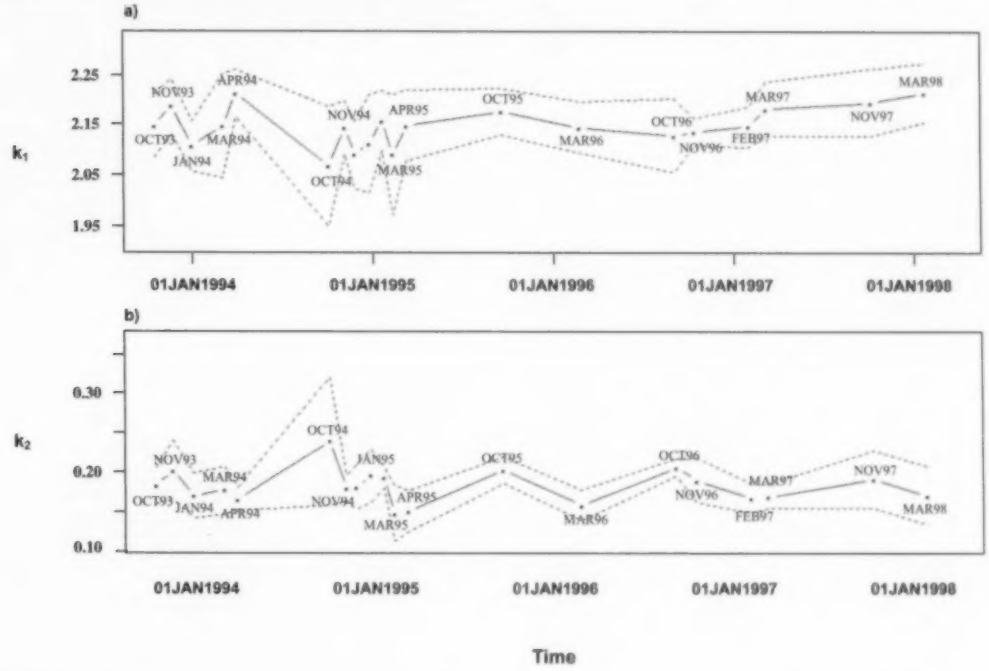
Fig. 5. Time series of the estimates of the selectivity parameters (*a*) k_1 (mean selection factor) and (*b*) k_2 (mean selection spread) in the investigation period. The points indicate a sampling period (survey). The 95% confidence limits are indicated with dotted lines. Labels for DEC1994, JAN1995, and FEB1995 are not presented.



and it was found that unimodal selectivity curves provided the most appropriate fits, in terms of minimum total deviance across all sets within a survey (Table 3). It was also found that fits to combined (pooled) data of the same curves led to severe overdispersion. Goodness-of-fit statistics were calculated based on the deviance for individual sets as well as for the combined data sets. It was found that the goodness-of-fit values were consistently higher for the individual sets than for the corresponding combined data set. Acceptable values for the goodness-of-fit for the combined data set were obtained in only four of 20 cases. However, 22% of the individual sets could not be used for estimation due to low catches.

Selectivity curves are presented on a transformed length (i.e., fish length divided by mesh size) unit scale. In order to better visualize the between-set variation within a survey, the mean selectivity curve along with the selectivity curves for the individual sets in the survey are presented on the same plot (Figs. 2 and 3).

Almost all surveys showed a high between-set variation (Fig. 3), which was accounted for when estimating the mean curves. The relative selectivity curves showed a very narrow shape compared with that of the pooled data, both for individual sets and for the mean curve.

The selectivity curves estimated when combining all sets by each survey resulted in substantial overdispersion and a higher estimated value of the spread (Fig. 4). The pooled data sets cover up for the variation in unobserved variables between sets. It is therefore only expected that the selectivity curve will accommodate this larger variation by attaining a larger spread.

A plot of the time series for the mean modal and the mean spread from the model output suggests a negative autocorrelation with a seasonal pattern (Fig. 5). From April 1995 onwards, the estimated mean selection factor seemed to stabilize (Fig. 5*a*) and the mean selection spread was systematically higher in the autumn than in the spring (Fig. 5*b*).

Discussion

In the present study, selectivity parameters were estimated using the method of Millar and Holst (1997). This method uses the maximum likelihood technique and thus provides estimates with a number of well-known and desirable properties such as asymptotic normal distribution, minimum variance, and unbiasedness. Although the underlying model assumes that the probability of a fish contacting the combined gear is Poisson distributed, it is rather robust to deviations from this assumption, provided that sufficient fish are caught in all length-classes and with all mesh sizes.

When estimating the selectivity curves to individual sets, several unimodal curves provided appropriate fits with only minor differences in log likelihood. A mixture of two normal scales (a bimodal curve) did not show any improvements in fits or resulted in unreasonable estimates. The overall best fits were achieved using a unimodal normal scale, indicating that the Atlantic herring were mainly caught by a single catch process. For the subsequent combining into a mean selectivity curve for each survey, it was necessary to use the same form of the selectivity curves for all sets within a survey. The random effects model accommodates, however, in-

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appropriate fits to individual sets, as these are likely to be encumbered with large variances and thus have reduced impact on the mean curve.

In trawl gear selectivity studies, it has become common practice to estimate the between-haul variation separately from the within-haul variance (Wileman et al. 1996). Fryer (1991) demonstrated that neglecting the between-haul variation could lead to severe underestimation of the variance of the parameters and could therefore result in unrealistically narrow confidence intervals. Estimation of between-set variation is not yet common in gillnet selectivity studies. The use of maximum likelihood techniques in the initial fitting of selectivity curves to individual gillnet sets allows the use of the same approach for estimating a mean selectivity curve and the between-set variance. The data used in this investigation revealed high between-set variation in almost all surveys. It was furthermore illustrated that estimation of selectivity curves for the combined (pooled) data sets led to substantial overdispersion. It is essential to all proper inference that the model used to describe the data reflect the structure and variability in the data in a realistic manner. The random effects model allows for both within-set and between-set variation. Testing of hypotheses and construction of confidence intervals based on this approach therefore represent an improvement compared with those based on pooled data. Caution should therefore be used both in considering between-set variation in the data and in estimating selectivity parameters using combined data sets.

Another advantage of the method used here is that it allows for the assessment of the power of the experiments, which can be very valuable in the planning of future trials. However, no power analysis was done here because the experimental designs were not initially based on any specifications of detectable differences and power levels.

The plot of the time series for the mean modal indicated stabilization from the survey from April 1995 onwards. It should be noted, however, that the sampling scheme in this "stable" period was characterized by a longer sampling interval than in the previous period, which could disguise fluctuations in the selection factor between the sampling periods.

The plot of the time series for the mean spread indicates autocorrelation with a seasonal pattern, at least after the estimation parameters were stabilized. The highest k_2 values in this period were found in autumn (October–November) and the lowest in spring (February–March). It was not possible to explain what caused this apparent seasonal pattern from the catch data alone. A possible explanation may be found in the population dynamics of the Rügen spring-spawning herring stock in the Sound, associated with seasonal changes in either reproduction strategy, condition factor (length–weight relationship of the individual fish), or fish behavioral factors. Rudstam et al. (1984) pointed out that the probability of catching a fish in a gill net can be separated into two components: (*i*) the probability of the fish encountering the net and (*ii*) the probability of the fish being caught and retained in the net. Therefore, gillnet selectivity will depend on both fish behavior and fish shape in relation to net proportions. Hay et al. (1986) found that several physiological factors affected selectivity including size, sex, and maturation (or gonad size). A difference in selectivity between locations, perhaps associated with biological differences between populations, was also found in these investigations. Finally, Hansson and Rudstam (1995) found that the physical conditions in the environment influenced the gillnet selectivity of herring in the Baltic Sea, with greater visibility significantly decreasing the likelihood of fish being caught in the gill nets. Having all these assumptions in mind, the differences that we observed in the selection pattern in the present study probably reflect changes in underlying fish behavior or seasonal changes in fish condition. It seems that the key factors for understanding these mechanisms are fish activity and distribution over time and space as well as fish condition, which all depend on environmental and biological factors influencing fish life history. Future research specifically designed to address these questions is needed to understand this phenomenon better.

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In situ survival and growth of three brook trout (*Salvelinus fontinalis*) strains subjected to acid conditions of anthropogenic origin at the egg and fingerling stages

Stephanie Lachance, Pierre Bérubé, and Michel Lemieux

Abstract: Tolerance to naturally acidic conditions of a Côte-Nord brook trout (*Salvelinus fontinalis*) strain (Arseneault strain, presumed acid tolerant) does not appear completely genetically mediated, since this tolerance was not evident when the fish were subject to acid conditions of anthropogenic origin. Three wild brook trout strains, at the egg and fingerling stages, were exposed in the field to waters acidified by atmospheric deposition of anthropogenic origin as well as to natural waters typical of the region. Although egg mortality was significantly higher in acid (61.0–85.6%) than in reference (6.3–20.8%) conditions, no differences between strains were noted. Residual density 1 year after stocking with fingerlings was significantly higher (by 56%) in the reference lake than in the acid lake, but differences between strains did not follow any clear tendency. While overall growth was greater in the acid environment, weight yield was lower except for the Arseneault strain. Variations pertaining to gonadosomatic index and percent mature individuals in both males and females seemed linked more to differences in growth than to chemical conditions. Developing a permanent reintroduction program with the Arseneault strain to counter losses of brook trout populations due to acid precipitation in Québec does not appear justified at this time.

Résumé : La tolérance aux conditions acides, limitantes mais naturelles, d'une lignée nord-côtière (lignée Arseneault, présumée tolérante à l'acidité) d'omble de fontaine (*Salvelinus fontinalis*) ne semble pas être seulement d'origine génétique puisque cette tolérance était peu évidente lorsque les poissons ont été soumis à des conditions acides d'origine anthropique. Aux stades oeufs et fretins, trois lignées sauvages d'omble de fontaine, ont été exposées en milieu naturel à des eaux acidifiées par les retombées atmosphériques d'origine anthropique ainsi qu'à des eaux aux caractéristiques naturelles pour la région. La mortalité des oeufs était significativement plus élevée en conditions acides (61,0 à 85,6%) qu'en conditions de référence (6,3 à 20,8%) pour les deux années d'expérimentation, mais il n'y avait aucune différence entre les lignées. La densité résiduelle 1 an après ensemencement des fretins était significativement plus élevée de 56% dans le lac de référence que dans le lac acide, mais les différences entre les lignées ne suivaient pas de tendances claires. La croissance était plus élevée en milieu acide, mais le rendement pondéral y était plus faible sauf pour la lignée Arseneault. Les variations concernant l'indice gonado-somatique des femelles et le pourcentage d'individus matures, tant chez les mâles que chez les femelles, semblaient liées aux différences de croissance plutôt qu'aux conditions physico-chimiques. Il n'apparaît pas justifié de développer la lignée Arseneault à des fins d'introduction dans les plans d'eau acidifiés qui soutenaient autrefois des populations d'omble de fontaine.

Introduction

According to Schindler (1988), massive fish mortality and the decline of salmonid fisheries are proof of the ecological damage caused by acid precipitation. In Québec, atmospheric deposition of sulphur and nitrogen oxides is associated with the disappearance of numerous fish populations, including several brook trout (*Salvelinus fontinalis*) populations (Tremblay and Richard 1993). Acid precipitation has become a primary environmental concern, not only in North America and Europe but also in Asia, Africa, and South America where sulphur and nitrogen oxide emissions are rapidly increasing as a result of continuing industrialization (Rodhe et al. 1995). It would appear that lakes are taking longer to recover than initially anticipated when the sulphur oxide emissions reduction program was first implemented (1985 in Canada and 1995 in the United States) (Jeffries 1997). Moreover, while sulphur oxide emissions have been on the decline, nitrogen oxide emissions have remained unchanged, and their acidifying capacity could increase as they accumulate in the environment (Jeffries 1997; Fenn et al. 1998).

A number of authors have examined the complex interactions between pH, Al, and Ca^{2+} that must be considered when attempting to determine the effects of acidification on

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S. Lachance and P. Bérubé.[1] Société de la faune et des parcs du Québec, Direction de la recherche sur la faune, 675 boul. René-Lévesque Est, 11ième étage, Boîte 92, Québec, QC G1R 5V7, Canada.
M. Lemieux. Société de la faune et des parcs du Québec, Direction de l'aménagement de la faune de la Mauricie, 605 ave. De la Station, Shawinigan, QC G9N 1V9, Canada.

[1]Author to whom all correspondence should be addressed.
e-mail: pierre.berube@fapaq.gouv.qc.ca

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fish (e.g., Mount et al. 1988*a*; Ingersoll et al. 1990*a*; Wood et al. 1990). Their findings reveal, in the case of brook trout, that pH < 5.2 concurrent with concentrations of filtrated Al > 200 $\mu g \cdot L^{-1}$ and Ca^{2+} < 2 $mg \cdot L^{-1}$ induces significant physiological stress on the species' ionoregulation and respiration capacity. These conditions can lead to recruitment failure (Haines 1981; Beggs and Gunn 1986).

Some lakes in Québec's Côte-Nord region are naturally acidic according to pH and concentrations of Al and Ca^{2+}. In theory, they should not support brook trout populations. However, regional biologists have found thriving populations (A. Gaudreault, Faune et Parcs Québec, Direction de l'aménagement de la faune de la Côte-Nord, 818 rue Laure, Sept-Îles, QC G4R 1Y8, Canada, personal communication). According to Rosseland and Skogheim (1987), natural selection in acidic environments produces populations that continue to manage well at low pH and high Al concentrations. Dupont (1991) explains that since the beginning of the industrial era, acidification by anthropogenic SO_4^{2-} has contributed to a pH decrease in Côte-Nord lakes of only 0.14 unit. This acidification level is the lowest recorded of all hydrographic regions surveyed within the Québec lake acidity monitoring network. Provincial studies have shown that, whereas catch per unit effort in the brook trout lakes of the Mauricie region is significantly correlated with pH and concentrations of Ca^{2+} and Al, this is not the case for Côte-Nord lakes (Tremblay 1991, 1993).

Our assumption is that brook trout populations in certain Côte-Nord lakes are highly tolerant to naturally occurring acidity, enabling them to maintain viable populations despite the limiting physicochemical properties of their environment. Rahel (1983) had also postulated the same theory in the case of a strain of yellow perch (*Perca flavescens*), which he deemed to be acid tolerant. This tolerance could be due to genetic factors and, if so, should become apparent if the trout are placed in an environment subjected to acidic deposition of anthropogenic sources. The possible genetic resistance of fish to acidity was noted by Gjedrem (1976), Rahel (1983), and Mills (1989). Tolerance could also be due to the presence in Côte-Nord waters of organic substances known to protect against the effects of acidity (Lacroix 1989; Campbell et al. 1992; Roy and Campbell 1997).

Comparisons among brook trout strains were made by Robinson et al. (1976) and Swarts et al. (1978) for tolerance to acidity. Their experiments were carried out in both laboratory and partially controlled environments reproducing extreme conditions similar to those caused by mine tailings. Their conclusion was that the brook trout's variable resistance to acidic conditions depended on genetic and environmental factors; therefore, they suggested using naturally resistant strains rather than attempting to develop such strains via selected hatchery-reared broodstock. Various performance comparisons between several naturally occurring strains of brook trout were also made by Jordahl and Benson (1987) and Van Offelen et al. (1994). In some instances, results showed great interindividual and interstrain variability with respect to tolerance to acidity, while in others, strains showed more or less similar resistance levels.

The main objective of this paper is to assess resistance to acidity of anthropogenic origin of a Côte-Nord brook trout strain and compare it with that of two other strains from areas affected by acid precipitation. The specific objectives are to compare, between an acidic and reference environment, (*i*) egg mortality up to emergence and (*ii*) survival, growth, gonadosomatic index (GSI), and percent maturity of individuals 1 year after stocking as fingerlings (0+).

Materials and methods

Origin of strains

The study protocol required comparing three strains of brook trout, one of which is presumed acid tolerant. In the present context, a strain is considered tolerant to acidity if it can survive and reproduce in an environment where pH is low (<5.2) but also where concentrations of Al are high (>200 $\mu g \cdot L^{-1}$) and those of Ca^{2+} are low (<2 $mg \cdot L^{-1}$). These three parameters do not act independently on fish health. For example, Ca^{2+} levels sufficient to support healthy development at circumneutral pH may prove inadequate under acidified conditions (Wood et al. 1990). Such a population was found in Arseneault Lake in the Sept-Îles Port-Cartier Wildlife Reserve (Fig. 1), where the three parameters presented levels far more severe than those usually considered critical for brook trout. Water samples taken in September 1996 at three different locations clearly illustrate the characteristics of this lake (Table 1). According to other analyses made in the spring of 1996, inorganic Al accounted for more than 85% of the lake's Al concentration. It is well known that this is the most toxic form of Al (Campbell et al. 1992). Broodstock was captured in September 1996 and kept in a government hatchery until the spring of 1998. The two other strains were obtained from the spawning of adult brook trout captured during the reproductive period at Banville Lake in the Laurentides Wildlife Reserve and at Bourassa Lake in the Mastigouche Wildlife Reserve (Fig. 1). The physiographic characteristics of these two lakes are typical of their respective regions and the Precambrian Shield and present nonlimiting physicochemical conditions for brook trout. The three strains of this study bear the names of their lakes of origin, i.e. Arsenault, Banville, and Bourassa.

Experiment 1: assessing egg mortality

Description of study sites

The experimental incubation sites selected for assessing egg mortality up to emergence were located at Olivier Lake in the Batiscan–Neilson Controlled Exploitation Zone (ZEC) and at Parke Lake in the Portneuf Wildlife Reserve, Québec (Fig. 1). A liming experiment conducted in 1994 at Olivier Lake offered relatively good, although temporary, results in terms of survival of stocked fingerlings (Faune et Parcs Québec, Direction de l'aménagement de la faune de Québec, Québec, unpublished data). This confirmed the fact that acid conditions in this lake were limiting trout survival. The Parke Lake spawning area acted as a reference site. Water samples were taken regurlarly during spring snowmelt to establish the physicochemical characteristics of both sites and better emphasize the extent of acidic episodes during this critical period. Integrated samples were also taken every 3 months at a depth of 0–5 m at the deepest end of the lakes immediately downstream from the incubation sites. The eight parameters used as indicators of water quality were pH (electrometric analysis), total alkalinity (titration with nitric acid), NO_2^-, NO_3^-, and SO_4^{2-} (automatic colorimetry), filtrated Al, Ca^{2+}, and Mg^{2+} (set using nitric acid and then analyzed by argon plasma spectrometry), and dissolved organic C (DOC) (electric conductivity following ultraviolet treatment to eliminate inorganic C). All analyses were made by the Centre d'expertise en analyse environnementale du Québec, Québec, Qué. In 1997, continuous temperature recorders (Minilog-TR,

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Fig. 1. Location of study sites (▲, Olivier Lake incubation site; ▲, Parke Lake incubation site; ▲, Blanc Lake; ▲, Eugène Lake) and spawner capture sites (1, Arseneault Lake; 2, Banville Lake; 3, Bourassa Lake) of the strains of brook trout studied.

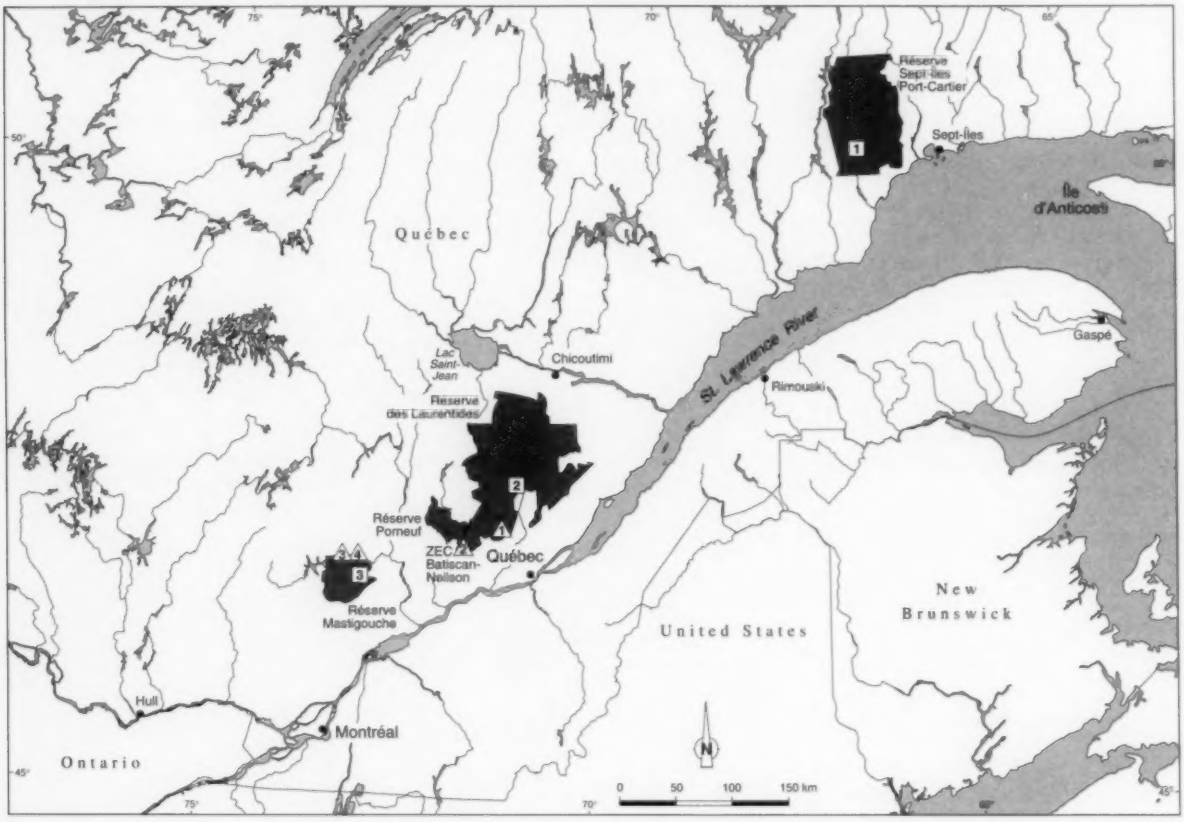

Table 1. Chemical characteristics of Arseneault Lake (Sept-Îles Port-Cartier Wildlife Reserve) water sampled in September 1996 during brook trout broodstock capture procedures.

Variable	Sample 1	Sample 2	Sample 3
Filtrated Al ($mg \cdot L^{-1}$)	0.29	0.27	0.23
Total alkalinity ($mg \cdot L^{-1}$)	0.2	0.3	0.3
Ca^{2+} ($mg \cdot L^{-1}$)	0.7	0.7	0.7
DOC ($mg \cdot L^{-1}$)	9.1	9.0	9.2
pH	4.8	4.9	4.9
SO_4^{2-} ($mg \cdot L^{-1}$)	0.5	1.0	1.0
Temperature (°C)	16	16	16

Vemco) were placed at both sites during the entire incubation period.

Spawning and incubation methods

The artificial reproduction and incubation methods described below have been used successfully for brook trout by Faune et Parcs Québec for several years (Boivin et al. 1990). The incubation boxes are made of rigid plastic pierced with numerous holes on all sides (Fig. 2). Protective layers of Astro-turf® are placed on the bottom and on the top, while layers with eggs are placed in between (Fig. 2). The boxes are completely submerged in such a way that the top of the box is slightly below the riverbed (Fig. 2). Each incubation site is chosen for appropriate depth, water velocity, and substrate diameter surrounding the box. After a hardening period of 60 min, freshly fertilized eggs were placed in six incubation boxes per incubation site, with two boxes for each strain and 1500 eggs (determined by volumetric method) divided evenly among 10 layers of Astro-turf® per box (Fig. 2). The gentle (in water using a feather) distribution of eggs on each layer was done in such a way as to maximize the separation between eggs. At Olivier Lake, although depth and velocity were adequate, gravel (diameter 9–50 mm) was added at the incubation site to aproximate the conditions at Parke Lake, which had been enhanced in 1994 and offered excellent conditions for spawning, incubation, and hatching. In fall 1997, 12 boxes instead of six were prepared for the Parke Lake site, as they were smaller and consisted of only five incubation layers for easier burial in the gravel. When the boxes were extracted from the riverbed in spring 1998, a box used for the Bourassa strain had to be discounted because holes had not been pierced on one of its sides and water circulation in the box risked being impaired. Assessment of the mortality of eggs up to emergence was carried out over two consecutive spring seasons, in 1997 and in 1998.

Assessing egg mortality

Dead egg counts were made on 12 June 1997 and 13 May 1998. The 1-month difference is due to extreme and contrasting weather conditions encountered in the springs of both years. However, water temperature at the time that the boxes were raised was the same in both years. Egg mortality up to emergence was established as the ratio of dead eggs counted on each layer in the spring to the estimated number of eggs on each layer the preceding fall. A

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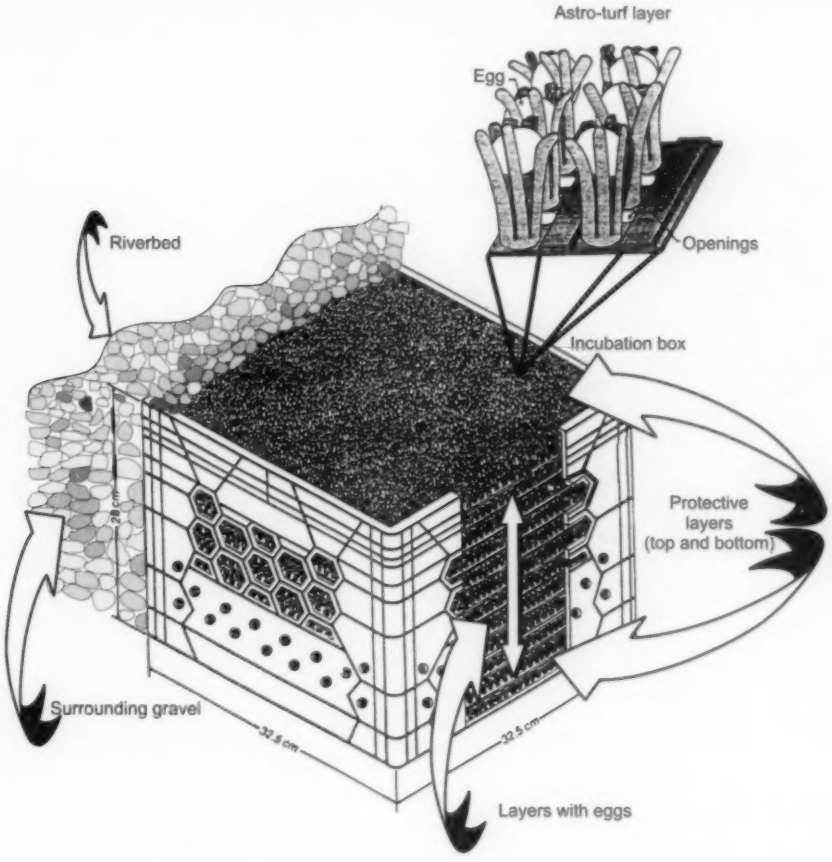
Fig. 2. Design of the incubation box with Astro-turf® layers used in experiments to evaluate egg mortality of the three brook trout strains.



multifactorial analysis of variance (ANOVA) with five factors (year, incubation site, strain, box, layer) was conducted to compare egg mortality using the following model:

$$\text{Egg mortality} = \text{Year} + \text{incubation site} + \text{strain} + (\text{year} \times \text{incubation site}) + (\text{year} \times \text{strain}) + (\text{incubation site} \times \text{strain}) + (\text{year} \times \text{incubation site} \times \text{strain}) + \text{box}(\text{year} \times \text{incubation site} \times \text{strain}) + \text{layer}(\text{box}(\text{year} \times \text{incubation site} \times \text{strain}))$$

The term box(year × incubation site × strain) is associated with the experimental units, while the term layer(box(year × incubation site × strain)) is associated with the sampling units, both terms being random. This was followed by multiple comparisons with a protected Fisher's least significant difference (LSD) test, where required. The normality of the data was verified against the Shapiro–Wilk statistic and the homogeneity of variances using an analysis of residuals. All tests were done using the SAS statistical analysis package (SAS 1996).

Experiment 2: assessing fingerling survival and growth

Fingerling marking method

Eggs obtained from the spawners of the three strains in 1996 were reared at the Baldwin Mills hatchery until they reached the fingerling stage in the autumn of 1997, i.e. at a length between 85 and 95 mm. To allow later identification, the fish were marked by fin clipping on 16 September 1997. They were first anesthetized with MS 222 (80 mg·L^{-1}). The adipose fin was excised from the Arseneault strain, the left pelvic fin from the Banville strain, and the right pelvic fin from the Bourassa strain. After marking, the fish from each strain were placed in separate basins and monitored over 10 days before stocking. This period showed that mortality due to marking stress was negligible (<1%) and that no infection had appeared.

Description of study sites

The experiments were conducted at Eugène Lake (area of 21.2 ha, mean depth of 5.2 m) and Blanc Lake (area of 24.9 ha, mean depth of 5.6 m) in the Mastigouche Wildlife Reserve (Fig. 1). In Blanc Lake, pH as well as Ca^{2+} and Al concentrations were at levels detrimental to the survival of brook trout. Apparently, no fish inhabit it and none have been caught in it since the government took over management of the wildlife reserve in 1973. In Eugène Lake, physicochemical parameters pose no threat to the survival of brook trout at any life stages, but there are no suitable spawning grounds able to sustain a population of this species. The lake harbours no other fish species. Before stocking, the outlets of both lakes were blocked with barriers made of solid wire mesh attached to deeply anchored steel rods and solidified with rocks to minimize losses by emigration. To characterize the lakes, a quarterly water quality monitoring program was implemented. Variables measured were the same as in experiment 1, and their level was assessed from an integrated sample (0–5 m) taken at the deepest end of the lake.

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Stocking method

All necessary precautions were taken to insure minimal stress to fish during transportation and stocking. The fish were distributed manually as evenly as possible over the surface area of the two lakes. Stocking density was set at a total of 150 individuals·ha^{-1}, which is 50 per strain. This density had been used with success in other studies on the survival, growth, and reproductive potential of various strains of brook trout stocked as fingerlings (Lachance and Magnan 1990*a*, 1990*b*). Prior to stocking, 50 arbitrarily chosen specimens were weighed (±0.05 g) and measured (total length, ±0.5 mm) individually for each strain in each lake to permit later appraisal of growth.

Assessing fingerling survival and growth

To estimate the residual population of each strain 1 year after stocking, a daily fishing effort of 20 gillnet-nights was used in the brook trout's preferred habitat (0–6 m). The effort lasted six nights at Blanc Lake and seven nights at Eugène Lake during the period from 31 August through 11 September 1998. The gill nets used (22.8 m in length by 1.8 m in width) consisted of six panels of 3.8 m and of stretched mesh measuring 25, 32, 38, 51, 64, and 76 mm. The nets were stationary, and the fishing effort lasted about 18 h, including the period from dusk until dawn.

Using the Leslie method (Ricker 1980), it was possible to estimate the initial population (N_0) found at the start of the fishing operation for each strain in each lake. The N_0 is defined as the ratio between the intercept point and the slope of the regression line between cumulative capture and daily capture. The variance associated with N_0 is that of the quotient from two random variables calculated using the linearization method. Using this variance, it was possible to compare N_0 between lakes and between strains using the maximum likelihood relationship statistic, which follows a χ^2 distribution. To correct for differences in lake area, density (N_0 per hectare) was used for comparisons.

To examine the growth of all three strains in both lakes, 100 individuals selected arbitrarily from among each strain in each lake were weighed (±0.05 g) and measured (total length, ±0.5 mm). Since mean length and weight of Bourassa strain individuals were significantly lower (ANOVA length: $F = 2035.34$, $p = 0.0001$; ANOVA log weight: $F = 2.65$, $p = 0.0001$) than mean length and weight of the other strains at the time of stocking and the growth of fish is not isometric, the use of statistical analyses to compare the size at capture 1 year after stocking of captured individuals between strains and lakes became impossible. Hence, three parameters were selected to characterize qualitatively the growth of fish: mean weight at capture, the percentage of weight increase, and the weight yield in terms of recovered biomass/stocked biomass for a given fishing effort.

Reproductive potential

Sex, total length (±0.5 mm), total weight (±0.05 g), gonad weight (±0.05 g), and the percentage of mature individuals were determined based on a sample of 100 individuals from each strain in each lake. The percentage of mature individuals by sex was compared using a logit model linking probable maturity to lake and strain factors (Agresti 1996; SAS 1996). This analysis was followed by multiple comparisons with an LSD test, where required. The GSI of mature individuals was calculated using the formula GSI = (gonad weight/body weight) × 100 (McCormick and Naiman 1984). Mean GSI values were compared for each sex separately using an ANOVA with two criteria (lake, strain) followed by an LSD multiple comparisons test, where required. The normality hypothesis was verified using the Shapiro–Wilk statistic and the homogeneity of variances using the Levene test. All tests were done using the SAS statistical analysis package (SAS 1996).

Results

Experiment 1: assessing egg mortality

Overall, during snowmelt, the water at the Olivier Lake incubation site was characterized by a pH in the 4.1–5.0 range, concentrations of filtrated Al in the 0.22–0.35 mg·L^{-1} range, and concentrations of Ca^{2+} in the 0.5–1.0 mg·L^{-1} range (Table 2). By contrast, at the same time of year, in the Parke Lake incubation area, pH was always >5.5, filtrated Al concentrations were <0.20 mg·L^{-1}, and Ca^{2+} concentrations usually remained around 2.0 mg·L^{-1} (Table 2). Throughout the year, the physicochemical conditions observed in the lakes downstream of the experimental incubation grounds were stable, and marked differences between the two lakes were noted for the three parameters of interest: pH and, more specifically, concentrations of filtrated Al and of Ca^{2+} (Table 3).

Mean egg mortality is presented in Table 4. There is a marked difference between mortality noted in acidic (61.0–83.6%) and nonacidic (6.3–20.8%) environments. The ANOVA detected a significant effect of the interaction between the factors year and incubation site ($F = 4.63$, $p = 0.0467$). Multiple comparisons tests clarified this effect by showing that egg mortality was significantly higher ($p = 0.0001$) at the acid site than at the reference site during the two years of the experiment but that this difference was smaller in 1998 than in 1997. More precisely, egg mortality at the acid site was significantly higher in 1997 than in 1998 ($p = 0.0001$) but remained the same at the reference site in both years ($p = 0.0783$). The strain factor had no significant effect on egg mortality irrespective of incubation conditions ($F = 0.3720$, $p = 0.6750$). It should be noted that very few yolk-sac fry were found dead and that dead eggs were not yet eyed. It would appear, therefore, that in the vast majority of cases, the effects of potential egg mortality factors are felt well before snowmelt, which usually occurs in April. Hatching at the Parke Lake site probably begins in March, given that mean incubation temperature varies between 0.5 and 1.0°C (Fig. 3). Hatching at the Olivier Lake site appears to have occurred later with respect to the Parke Lake site, since in the two years of the experiment at that site, newly hatched fry (89 in 1997 and 187 in 1998) as well as dead fry (157 in 1997 and 233 in 1998) trapped by their chorion were found in the boxes. At the Parke Lake site, only three newly hatched fry were observed and no dead fry were found during the two years of the experiment. Survival to emergence only was taken into account, as postincubation survival was not assessed. Worthy of note is the fact that emergent fry were not found in the vicinity of the acid incubation site, yet were abundant and very active when the boxes at the reference site were extracted from the riverbed.

Experiment 2: assessing fingerling survival and growth

Water quality

As opposed to Eugène Lake, concentrations of filtrated Al measured at Blanc Lake were five to six times greater, concentrations of Ca^{2+} twice as low, pH lower by 1.5 units, and alkalinity often 30 times lower (Table 5). Physicochemical conditions at Blanc Lake, it should be recalled, do not support natural brook trout populations.

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Table 2. Chemical characteristics of water sampled at spawning sites during snowmelt between the 25 March and 14 May in 1997 and 1998.

	1997						1998							
Variable	25 Mar.	9 Apr.	22 Apr.	1 May	6 May	14 May	25 Mar.	2 Apr.	9 Apr.	15 Apr.	23 Apr.	1 May	16 May	14 May
Olivier Lake spawning site (acid)														
Filtrated Al ($mg \cdot L^{-1}$)	0.23	0.26	0.28	0.35	0.26	0.27	0.22	0.31	0.29	0.34	0.30	0.30	0.33	0.25
Total alkalinity ($mg \cdot L^{-1}$)	<0.1	0.2	<0.1	<0.1	0.1	<0.1	2.0	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	0.2
Ca^{2+} ($mg \cdot L^{-1}$)	1.6	1.0	1.0	0.7	0.7	0.5	1.4	0.7	0.9	0.7	0.5	0.6	0.6	0.8
DOC ($mg \cdot L^{-1}$)	7.2	5.2	5.2	6.2	5.2	5.4	5.0	5.9	5.2	6.4	5.9	5.1	6.5	5.7
Mg^{2+} ($mg \cdot L^{-1}$)	0.3	0.2	0.2	0.1	0.1	0.1	0.3	0.1	0.2	0.1	0.1	0.1	0.1	0.1
NO_2^- and NO_3^- ($mg \cdot L^{-1}$)	0.08	0.21	nd	nd	nd	nd	0.08	0.06	0.06	<0.02	0.03	0.02	<0.02	<0.02
pH	4.1	nd	4.4	4.7	4.9	4.8	5.8	4.7	5.0	4.8	4.8	4.9	4.6	4.9
SO_4^{2-} ($mg \cdot L^{-1}$)	11.0	6.5	2.5	2.5	2.0	3.0	2.3	2.7	2.4	2.1	1.7	2.0	1.6	1.5
Parke Lake spawning site (reference)														
Filtrated Al ($mg \cdot L^{-1}$)	0.12	0.12	0.15	0.19	0.22	0.14	0.11	0.19	0.17	0.17	0.14	0.10	0.09	0.09
Total alkalinity ($mg \cdot L^{-1}$)	2.9	1.9	1.8	1.0	0.9	1.3	3.0	1.2	1.7	1.8	1.4	2.0	nd	2.6
Ca^{2+} ($mg \cdot L^{-1}$)	2.1	2.2	2.1	1.7	1.4	1.4	2.2	2.0	2.1	2.1	1.4	1.9	1.9	2.0
DOC ($mg \cdot L^{-1}$)	5.9	3.5	5.8	5.8	5.4	4.7	4.8	7.3	7.0	6.4	5.5	4.8	4.7	4.8
Mg^{2+} ($mg \cdot L^{-1}$)	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3
NO_2^- and NO_3^- ($mg \cdot L^{-1}$)	0.17	0.21	nd	nd	nd	nd	0.13	0.17	0.15	<0.02	0.10	0.09	0.05	1.91
pH	5.8	5.8	5.6	5.5	5.5	5.7	6.0	5.6	5.6	5.7	5.6	6.0	6.0	6.0
SO_4^{2-} ($mg \cdot L^{-1}$)	1.5	2.5	5.0	2.8	4.0	2.0	3.4	3.6	3.7	5.7	2.6	5.9	4.7	1.5

Note: nd, not determined.

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Table 3. Chemical characteristics of water sampled in the lakes adjacent to the experimental spawning sites (integrated sample 0–5 m).

	Olivier Lake (acid)						Parke Lake (reference)					
Variable	Nov. 1996	Apr. 1997	Aug. 1997	Oct. 1997	Mar. 1998	May 1998	Nov. 1996	Apr. 1997	Aug. 1997	Oct. 1997	Mar. 1998	May 1998
Filtrated Al ($mg \cdot L^{-1}$)	0.19	0.28	nd	0.17	0.20	0.22	0.05	0.05	nd	0.03	0.07	0.07
Total alkalinity ($mg \cdot L^{-1}$)	0.3	0.3	0.2	0.4	0.9	0.4	1.2	1.1	1.6	nd	1.4	1.3
Ca^{2+} ($mg \cdot L^{-1}$)	1.0	1.0	0.8	1.0	1.0	0.8	1.5	1.6	1.5	1.5	1.9	1.4
DOC ($mg \cdot L^{-1}$)	4.7	6.2	4.3	4.2	4.3	4.3	2.6	3.0	2.4	2.4	3.0	2.6
Mg^{2+} ($mg \cdot L^{-1}$)	0.1	0.2	0.1	0.1	0.2	0.1	0.3	0.3	0.3	0.3	0.3	0.3
NO_2^- and NO_3^- ($mg \cdot L^{-1}$)	0.04	0.14	nd	0.03	0.06	0.02	0.14	0.25	nd	0.15	0.23	0.11
pH	5.4	6.0	5.5	6.0	5.3	5.2	6.1	5.9	6.4	6.2	5.8	6.1
SO_4^{2-} ($mg \cdot L^{-1}$)	3.0	2.5	1.0	17	2.1	2.0	3.0	5.5	2.5	2.0	3.8	3.5

Note: nd, not determined.

Table 4. Mean percent mortality of eggs of the three strains of brook trout incubated for approximately 30 weeks in experimental spawning sites for both years of testing.

		1997			1998		
Spawning site	Strain	No. of boxes	No. of layers	Mean % mortality per layer (±1 SD)	No. of boxes	No. of layers	Mean % mortality per layer (±1 SD)
Olivier Lake (acid)	Arseneault	2	20	80.1 (±20.5)	2	20	67.5 (±8.2)
	Banville	2	20	83.6 (±14.1)	2	20	61.0 (±11.8)
	Bourassa	2	20	77.2 (±18.0)	2	20	71.2 (±18.0)
	All strains	6	60	80.3*a** (±17.6)	6	60	66.5*a** (±13.7)
Parke Lake (reference)	Arseneault	2	20	6.3 (±12.1)	4	20	20.8 (±22.6)
	Banville	2	20	7.7 (±13.5)	4	20	6.3 (±8.0)
	Bourassa	2	20	11.0 (±10.6)	3	15	10.7 (±12.7)
	All strains	6	60	8.3*b* (±12.1)	11	55	12.7*b* (±16.8)

Note: For a significant ANOVA ($p < 0.05$) within a column, means followed by the same letter are not significantly different (LSD multiple comparisons test, $p = 0.05$). For a significant ANOVA ($p < 0.05$) within a row, means followed by an asterisk are significantly different (LSD multiple comparisons test, $p = 0.05$).

Fingerling survival

Overall, brook trout residual density (N_0 per hectare) 1 year after stocking in Blanc Lake (acid) was significantly lower ($\chi^2 = 15.70$, $p < 0.05$) than in Eugène Lake (reference) by 56% (Table 6). Percent return of the stocked trout was 62% in Eugène Lake and 27% in Blanc Lake (Table 6). In Blanc Lake, residual densities of the Arseneault and Bourassa strains were significantly higher ($\chi^2 = 20.13$, $p < 0.05$) than that of the Banville strain (Table 6). However, these differences remain very slight. In Eugène Lake, no significant difference was detected between residual densities after stocking of all three strains, despite the fact that the Banville strain showed a strong tendency towards greater returns and a higher residual density. The absence of significance of this tendency could be due in major part to the great variance associated with the estimation of N_0 for the Arseneault strain in that lake. Decline in the number of fish caught from day to day was much more irregular in Eugène Lake than in Blanc Lake for all three strains, but especially so for the Arseneault strain.

Growth and weight yield

The mean weight of Arseneault strain captures was lower than that of the other strains by 45% in Eugène Lake and 21% in Blanc Lake (Table 7). For all strains, the percentage of weight increase in Blanc Lake was generally higher than in Eugène Lake. This was mainly evident in the Arseneault strain whose percentage of increase was twice as high in Blanc Lake. In both lakes, the greatest weight increase was recorded by the Bourassa strain and the smallest by the Arseneault strain (Table 7). Weight yield for the Banville and Bourassa strains was lower in Blanc Lake than in Eugène Lake by 44 and 35%, respectively. Conversely, the weight yield for the Arseneault strain was 30% higher in Blanc Lake than in Eugène Lake.

Reproductive potential

No difference in percentage of mature individuals for each sex was recorded between the acid and the reference lake. Less than 45% of females captured were mature (Table 8). Percent mature females was significantly lower for the Arseneault strain than for the two other strains ($F = 8.92$, $p = 0.0002$) (Table 8). The percentage of mature males was upwards of 85% in all cases, with no significant difference between strains (Table 8). There was no difference between acid and reference lakes for mean GSI of mature females. However, it was significantly lower for the Bourassa strain with respect to the Arseneault and Banville strains in both lakes ($F = 19.82$, $p = 0.001$). The ANOVA revealed that lake and strain factors had significant separate effects on the mean GSI of mature males. Overall, the mean GSI of mature males in Blanc Lake was lower than that of males in Eugène

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Fig. 3. Mean daily temperatures registered during the brook trout incubation period (autumn 1997 through spring 1998) at the two experimental incubation sites (solid line, Olivier Lake; broken line, Parke Lake).

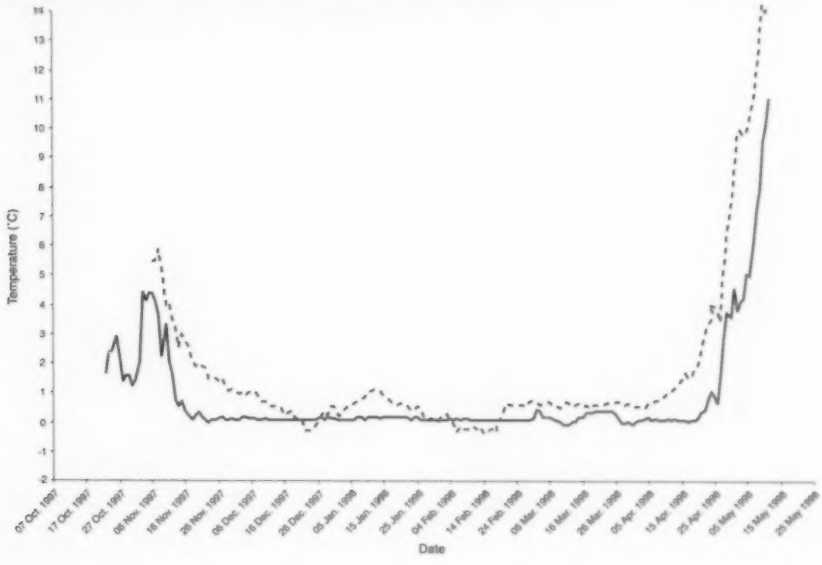

Table 5. Chemical characteristics of water sampled in lakes used in the study of brook trout fingerling survival and growth (integrated sample 0–5 m).

	Blanc Lake (acid)							Eugène Lake (reference)						
Variable	Oct. 1996	Feb. 1997	May 1997	Aug. 1997	Oct. 1997	Feb. 1998	May 1998	Oct. 1996	Feb. 1997	May 1997	Aug. 1997	Oct. 1997	Feb. 1998	May 1998
Filtrated Al ($mg \cdot L^{-1}$)	0.17	0.21	0.20	0.12	0.08	0.18	0.21	0.03	0.04	0.04	0.02	<0.01	0.03	0.04
Total alkalinity ($mg \cdot L^{-1}$)	<0.1	0.3	<0.1	0.1	<0.1	<0.1	0.1	3.3	3.6	nd	3.5	3.8	3.6	3.3
Ca^{2+} ($mg \cdot L^{-1}$)	1.2	1.1	1.0	1.0	1.0	1.2	1.0	2.4	2.3	2.3	2.2	2.2	2.4	2.3
DOC ($mg \cdot L^{-1}$)	5.7	5.6	4.7	3.6	3.5	5.5	4.8	4.0	3.7	4.3	4.2	3.6	3.8	3.5
Mg^{2+} ($mg \cdot L^{-1}$)	0.3	0.3	0.2	0.2	0.3	0.3	0.2	0.3	0.4	0.3	0.3	0.3	0.4	0.3
NO_2^- and NO_3^- ($mg \cdot L^{-1}$)	<0.02	0.02	<0.02	<0.02	<0.02	0.46	<0.02	<0.02	0.02	0.04	<0.02	<0.02	0.05	0.03
pH	5.0	4.9	5.0	5.3	5.3	5.0	4.9	6.6	6.3	6.5	6.7	6.7	6.3	6.4
SO_4^{2-} ($mg \cdot L^{-1}$)	3.5	3.5	4.0	3.0	3.5	1.0	3.5	2.5	3.5	4.0	3.5	4.0	2.5	2.8

Note: nd, not determined.

Table 6. Residual density, as estimated by the Leslie method, of brook trout 1 year after stocking as fingerlings in experimental lakes.

	Blanc Lake (acid)				Eugène Lake (reference)			
	Arseneault	Banville	Bourassa	All strains	Arseneault	Banville	Bourassa	All strains
Stocking density ($no. \cdot ha^{-1}$)	50	50	50	150	50	50	50	150
Residual density after 1 year ($N_0 \cdot ha^{-1}$ (±1 SD))	15*a* (±0.4)	12*b* (±0.4)	14*a* (±0.7)	41* (±2)	28a (±16)	44*a* (±8)	27*a* (±3)	93* (±13)
% return after 1 year ($N_0 \cdot$no. stocked^{-1} × 100)	30	24	28	27	56	88	54	62

Note: For a significant χ^2 ($p < 0.05$) within a lake, densities followed by the same letter are not significantly different. For a significant χ^2 ($p < 0.05$) between lakes, densities followed by an asterisk are significantly different.

Lake ($F = 6.47$, $p = 0.0116$). Moreover, for mature males as a whole, the mean GSI was significantly lower for the Bourassa strain than for the Banville strain, which was itself lower than for the Arseneault strain ($F = 8.46$, $p = 0.0003$). However, these differences seem very minor.

Discussion

The high egg mortality at the Olivier Lake incubation site is most likely due to the prevailing physicochemical conditions. While water quality measurements may have only

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Table 7. Mean weight and yield by weight of the three strains of brook trout 1 year after stocking as fingerlings in experimental lakes.

	Blanc Lake (acid)			Eugène Lake (reference)		
	Arseneault	Banville	Bourassa	Arseneault	Banville	Bourassa
Weight at stocking (Oct. 1997, n = 50) (g ± 1 SD)	7.8*a* (±3.8)	6.8*a* (±1.9)	5.3*b* (±1.2)	6.6*a* (±2.8)	7.3*a* (±1.9)	5.1*b* (±1.3)
Weight at capture 1 year later (Sept. 1998, n = 100) (g ± 1 SD)	116.5 (±42.4)	155.7 (±44.5)	139.4 (±35.3)	72.8 (±27.2)	144.0 (±42.9)	121.5 (±38.6)
% increase in weight	2040	2203	2545	1001	1886	2301
Total biomass stocked (kg)	9.7	8.4	6.6	7.0	7.7	5.4
Total biomass recuperated for 120 gillnet-nights (kg)	42.7	47.0	51.0	21.5	76.9	63.6
Yield by weight (biomass recuperated/biomass stocked)	4.4	5.6	7.8	3.1	10.0	11.9

Note: For a significant ANOVA ($p < 0.05$), means followed by the same letter are not significantly different (LSD multiple comparisons test, $p = 0.05$).

Table 8. Weight, total length, percent maturiy, and GSI of the three strains of brook trout 1 year after stocking as fingerlings in the experimental lakes.

	Blanc Lake (acid)			Eugène Lake (reference)		
	Arseneault	Banville	Bourassa	Arseneault	Banville	Bourassa
Females						
No. examined	49	45	59	41	27	41
Mean weight (g ± 1 SD)	106.5 (±37.3)	137.9 (±37.5)	137.2 (±37.0)	65.3 (±25.0)	123.8 (±37.3)	118.2 (±35.2)
Mean total length (mm ± 1 SD)	219 (±23)	239 (±20)	237 (±21)	180 (±23)	232 (±19)	226 (±22)
% mature	4.1*a*	28.9*b*	23.7*b*	2.4*a*	44.4*b*	26.8*b*
Mean GSI of mature females (% ± 1 SD)	4.1*a* (±3.0)	5.1*a* (±1.4)	2.6*b* (±0.9)	4.8*a* (±0)	5.0*a* (±1.5)	2.8*b* (±0.8)
No. mature	2	12	14	1	11	9
Males						
No. examined	40	46	26	44	66	36
Mean weight (g ± 1 SD)	137.2 (±42.1)	175.4 (±46.8)	151.0 (±32.8)	81.5 (±29.5)	156.0 (±41.3)	139.3 (±38.9)
Mean total length (mm ± 1 SD)	234 (±25)	253 (±19)	238 (±20)	199 (±23)	252 (±21)	238 (±21)
% mature	92.5*a*	87.0*a*	96.3*a*	95.4*a*	89.4*a*	86.1*a*
Mean GSI of mature males (% ± 1 SD)	3.5 (±0.7)	3.2 (±0.7)	2.9 (±0.9)	3.1 (±0.8)	3.0 (±0.7)	2.6 (±1.1)
No. mature	37	39	26	42	59	28

Note: For a significant effect in the logit model ($p < 0.05$) for each sex taken separately, percent mature values followed by the same letter are not significantly different (LSD multiple comparisons test, $p = 0.05$). For a significant ANOVA ($p < 0.05$) for females, mean GSI values followed by the same letter are not significantly different (LSD multiple comparisons test, $p = 0.05$).

been taken during snowmelt at both sites, they still certainly emphasize and outline the strong differences observed. Since 1989, pH spot-checks, carried out by Faune et Parcs Québec at the Olivier Lake incubation site as part of other studies, have yielded levels of 4.9, 4.6, and 4.5 in July, August, and September, respectively. Moreover, measurements made in the lake downstream from the site revealed that Ca^{2+} and Al concentrations in Olivier Lake remain close to critical values for the survival of brook trout year-round. It is a known fact that acid conditions in lakes can be less restrictive than those in tributaries, owing to the greater dilution capacity of lakes (Schindler 1988). In addition, because of the biochemical processes involved in primary production and degradation, lakes have a buffering capacity (Schindler et al. 1986; Jeffries 1997) that streams do not. These facts suggest that the physicochemical conditions in the acid tributary stream are restrictive year-round.

Incubation temperatures recorded at both sites were cold, especially at Olivier Lake. Cold temperatures usually extend the developmental period without having an impact on the survival of salmonid eggs (Dwyer 1987; Marten 1992). At 0.9°C, the survival rate of lake trout (*Salvelinus namaycush*) eggs is 86%, even though development takes 40 days longer than eggs incubated at 8°C (Dwyer 1987). Such conditions appear frequent at brook trout spawning sites in Québec. At the hatchery operated by Faune et Parcs Québec in the Laurentides Wildlife Reserve, incubation takes place at temperatures close to 0°C, and the survival rate for the Banville strain there exceeds 80%. It therefore seems very unlikely that egg survival was adversely affected by temperature in this case.

The fact that egg mortality occurs very early in the developmental stage, before the embryonic stage, in the majority of cases corroborates the findings by many authors (e.g.,

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Menendez 1976; Wood et al. 1990) that freshly fertilized eggs are very sensitive to pH levels below 5.2, particularly when Ca^{2+} concentrations are below 2 $mg \cdot L^{-1}$. In fact, at pH 4.8, it would appear that Ca^{2+} concentrations in the 8 $mg \cdot L^{-1}$ range are necessary to compensate for the negative effects of acid on transmembrane exchanges (Wood et al. 1990). Also, weakening of the chorion by acid conditions would interfere with respiration and osmoregulation (Haines 1981). Levels of pH below 4.5 in combination with Ca^{2+} concentrations below 2 $mg \cdot L^{-1}$ cause 100% mortality in exposed brook trout eggs (Hunn et al. 1987). The similar response among strains suggests that tolerance to acid conditions of the Côte-Nord brook trout is not totally genetically mediated, at least where these life stages are concerned. These findings conflict with Gjedrem's (1976) studies on brown trout (*Salmo trutta*), which revealed mainly genetic differences in tolerance between strains during incubation. He explained that to achieve this tolerance level, selection had to occur over many generations to be fairly homogeneous. On the Côte-Nord, where pH levels below 5 and concentrations of Ca^{2+} below 1 $mg \cdot L^{-1}$ and filtrated Al below 200 $mg \cdot L^{-1}$ have in all likelihood prevailed for a very long time, owing to their natural origin, hundreds of years of natural selection has been taking place. Concentrations of DOC in Arseneault Lake are in the order of 9 $mg \cdot L^{-1}$. The role of DOC as a protective agent against the adverse effects of toxic metals, especially Al, has been well documented (Lacroix 1989; Campbell et al. 1992; Roy and Campbell 1997). However, toxicity to the early life stages during incubation seems to be due to low pH levels and low Ca^{2+} concentrations rather than elevated Al (Menendez 1976; Wood et al. 1990). The fact that the Arseneault strain does not tolerate acid conditions during incubation is therefore puzzling. The protective effect of various organic acids warrants further investigation, following the example of Roy and Campbell (1997) with fulvic acid.

It is difficult to explain the significantly higher mortality of eggs in acid conditions in 1997 than in 1998, on the sole basis of physicochemical parameters recorded at the experimental incubation site. However, it must be noted that on 9 November 1996, immediately after the installation of the incubation boxes, a major flood occurred that may have also had a further impact on the survival of the eggs, on both a physical (stronger than usual mechanical mixing) and a chemical level (acid episode induced by runoff from heavy rain).

Embryo development at the acid incubation site appears to have been slower than at the reference site. Moreover, at the acid site, many embryos were found dead in the chorion. Differences in development rates could be explained in part by differences of a few degrees in temperature between experimental sites at the onset of the incubation period. However, the combination of delayed development and hatching difficulty is a known indicator of the effects of low pH, Ca^{2+} concentration, and temperatures on salmonid fish eggs having survived to the embryo stage (e.g., Haines 1981; Jordahl and Benson 1987).

The low number of brook trout captured 1 year after stocking in the acid lake confirms the findings that combined pH, Ca^{2+}, and Al conditions of the same order of magnitude as those recorded in Blanc Lake compromise survival (e.g., Ingersoll et al. 1990*a*; Marschall and Crowder 1996). The absence of marked differences in the return rates of the different strains suggests that the survival capacity of the Arseneault brook trout strain is not due to genetic adaptation. This result is confirmed by the work of Ingersoll et al. (1990*b*) and Van Offelen et al. (1994) but runs counter to the conclusions drawn by Robinson et al. (1976) and Swarts et al. (1978) who attributed a nonnegligible fraction of genetic adaptation to increased resistance of certain strains to extremely low pH. The protective role of DOC, which acts at the fingerling stage (e.g., Simonin et al. 1993; Roy and Campbell 1997), could be an important factor for the survival of the Côte-Nord brook trout populations. DOC concentrations in Arseneault Lake, about 9 $mg \cdot L^{-1}$, are about 50% higher than in Blanc Lake. Lacroix et al. (1990) have reported that a DOC concentration of about 5 $mg \cdot L^{-1}$ protects against the effects of Al at a concentration of 0.36 $mg \cdot L^{-1}$, even though the Ca^{2+} concentration is below 1 $mg \cdot L^{-1}$. These conditions induce chelation, which significantly reduces the fraction of available Al ions, a huge stress factor for fish. Existing environmental conditions rather than genetically mediated tolerance would be responsible for the survival of these life stages on the Côte-Nord.

The studies by Lachance and Magnan (1990*a*, 1990*b*), who compared growth and the recovery rate by angling after stocking of domestic, hybrid, and wild strains of brook trout, provide a good reference point for the growth and reproductive potential noted in the present study. An identical stocking protocol in the same wildlife reserve as well as the same fish strain from Banville Lake were used.

In our study, the weight at stocking of the Banville strain was lower than that recorded by Lachance and Magnan (1990*a*). In contrast, however, individuals from that strain caught 1 year after stocking weighed 25–30% more in our experiment than those caught by Lachance and Magnan (1990*b*) under similar conditions. In laboratory tests, acid conditions usually impair the growth of brook trout (Ingersoll et al. 1990*a*; Marschall and Crowder 1996). But our study revealed that the growth of brook trout in the acid lake was greater than that of brook trout in the reference lake. If we assume the presence of similar food resources, this result could be explained by a reduction in competitive pressure on fish having survived and acclimated, owing to the lower density of fish. This response was reported by Frenette and Richard (1986) as well as by Saint-Pierre and Moreau (1986) for brook trout. At the hatchery, prior to stocking, growth of the Arseneault strain was excellent, better in fact than for the Bourassa strain, according to size at stocking data for both strains. This suggests that the low growth rate of the Arseneault strain at the reference site in relation to that of the other strains is due to competitiveness rather than to a physiological difference in growth rate. In the acid environment, the slow growth is not as evident, as the mean weight of the Arseneault strain is greater than that in the reference environment by 45 g. Moreover, while the mean weight of the Arseneault strain is 23 g lower than that of the Bourassa strain and 39 g lower than that of the Banville strain in the acid environment, in the reference environment, the difference is nearly double at 49 and 71 g, respectively. Because residual densities of brook trout after stocking in the acid lake were 50% lower than in the refer-

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ence environment, it is impossible to confirm whether or not the difference in growth among individuals from the Arseneault strain in the acid and reference environments is a consequence of better adaptation to acid conditions. Lesser competitive pressure among strains due to a low total density could also explain this result. Nevertheless, the Arseneault strain was the only strain with a higher weight yield in the acid environment (4.4 kg recovered·kg stocked^{-1}) than in the reference environment (3.1 kg recovered·kg stocked^{-1}).

Overall, for both males and females, the percentage of mature individuals was not significantly different between acid and reference lakes. It appears therefore that this parameter is not appreciably affected by the acidity level measured in Blanc Lake. According to McCormick and Naiman (1984), size rather than age is more important in determining sexual maturation of brook trout. Furthermore, Saint-Pierre and Moreau (1986) showed that there is no difference among acid and nonacid lakes with respect to size of brook trout at sexual maturity. In the Mastigouche Wildlife Reserve, wild female brook trout in lakes reach maturity when they have grown to 180 mm (P. Magnan, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, QC G9A 5H7, Canada, personal communication). Because most individuals had attained this critical length during the summer period, the absence of an overall difference, concerning the percentage of mature individuals, between strains and lakes for both sexes is not suprising. The percentage of mature females from the Banville strain in the reference lake (44.4%) is much higher that that reported by Lachance and Magnan (1990*b*) under very similar conditions (3.0%). The greater size of the brook trout from the Banville strain in this study could explain this difference. The percentage of mature males from the Banville strain is very similar to that obtained by Lachance and Magnan (1990*b*), i.e. 93%. Interstrain differences in percent maturity can also be explained by size. The lower percentage of mature females in the Arseneault strain would be a response to this strain's slower growth. No strain-dependent effect on percentage of mature males was detected; this is consistent with the fact that males reach maturity at a smaller size than do females (McCormick and Naiman 1984).

Mean GSI of mature females from the Bourassa strain and, to a lesser extent, mean GSI of males are significantly lower than those of the other strains. The lower reproduction effort invested by this strain at age 1+ could be due to differences in size in the first months of life, as indicated by the mean size at stocking, or to genetic differences. However, acid conditions do not explain the differences between strains, at least not in the case of females, since trends are the same in both lakes. These results contrast those obtained by Saint-Pierre and Moreau (1986) who postulated that the GSI of mature males and females is higher in acid lakes than in reference lakes. Other studies have shown the adverse effect of acid conditions on vitellogenesis, fecundity, and egg viability (Mount et al. 1988*a*, 1988*b*), but there is scant information on the impact of acidity on GSI.

The results of this study clearly show the impact of acid conditions on brook trout at two life stages. The Arseneault strain, presumed tolerant to acid conditions, did not perform noticeably or substantially better than the other strains. In fact, during incubation at the acid site, the vast majority of eggs from the Arseneault strain died in the first stages of development, as did eggs from the other strains. A year after stocking in the acid lake, fish from this strain showed a slightly greater residual density than that of one of the two other strains. Their growth was delayed with respect to the other strains, but this difference was less important in the acid environment. In the absence of interstrain competition, growth of the Côte-Nord strain could have been similar to that of the other strains, based on the excellent growth observed at the hatchery prior to stocking. Further studies on growth in the absence of other strains could possibly justify developing this strain eventually for put–grow–take stocking purposes in acid lakes, considering its better yield by weight in acid rather than reference conditions, even in the presence of other strains. However, in light of the present results, developing a permanent reintroduction program for the Arseneault strain to counter losses of brook trout populations due to acid precipitation in lakes of south-central Québec does not appear advisable or justified at this time.

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Nutrient–chlorophyll relationships: an evaluation of empirical nutrient–chlorophyll models using Florida and north-temperate lake data[1]

Claude D. Brown, Mark V. Hoyer, Roger W. Bachmann, and Daniel E. Canfield, Jr.

Abstract: Nutrient–chlorophyll (CHL) relationships were developed using a large data set collected in Florida over the last 10 years consisting of monthly total phosphorus (TP), total nitrogen (TN), and CHL concentrations from 360 lakes. The precision of these and five additional published relationships was examined. The 95% confidence interval for the best available TP–CHL model is 30–325% of the calculated CHL value. Analysis of associated Florida monthly nutrient and CHL data indicate that the TP–CHL relationship is sigmoid, although a linear response is found for TP concentrations in the range of 3–160 $\mu g \cdot L^{-1}$. The maximum CHL responses for a sigmoid curve and straight line are similar for TP concentrations of 3–100 $\mu g \cdot L^{-1}$. Both relationships describe P limitation when the CHL response falls on or near the line and provide a benchmark to evaluate other limiting or colimiting factors that are indicated when the CHL response falls below the line. Florida and global data are similar, exhibiting a lessening of slope above a TP concentration of 100 $\mu g \cdot L^{-1}$. A global median line is derived from a large population of lake data for use in general lake management.

Résumé : Nous avons examiné les relations entre les nutriants et la chlorophylle à partir d'une grande base de données recueillies en Floride depuis 10 ans, représentant les concentrations moyennes de phosphore total (PT), d'azote total (NT) et de chlorophylle (CHL) dans 360 lacs. Nous avons examiné la précision de ces relations et de cinq autres études publiées sur le sujet. L'intervalle de confiance de 95% pour le meilleur modèle PT–CHL correspond à 30% à 325% de la valeur calculée de CHL. L'analyse des données mensuelles associées (pour la Floride) sur les nutriants et la CHL indique que la relation PT–CHL est sigmoïde, tandis qu'on observe une réponse linéaire pour les concentrations de PT dans la fourchette de 3 à 160 $\mu g \cdot L^{-1}$. La réponse maximum de CHL pour la courbe sigmoïde et la ligne droite sont similaires dans la plage de concentration de PT de 3–100 $\mu g \cdot L^{-1}$. Les deux relations révèlent une limitation par le phosphore quand la réponse de CHL tombe sur la ligne ou à proximité, et fournissent un jalon permettant d'évaluer d'autres facteurs limitants ou colimitants qui sont signalés par le fait que la réponse de CHL tombe au-dessous de la ligne. Les données obtenues en Floride et ailleurs dans le monde sont similaires, et montrent un abaissement de la courbe au-dessus d'une concentration de PT de 100 $\mu g \cdot L^{-1}$. Nous avons calculé une ligne médiane globale à partir d'un vaste corpus de données sur les lacs, ce qui peut servir à la gestion d'ensemble des lacs.

[Traduit par la Rédaction]

Introduction

During the last 60 years, several researchers have demonstrated a strong correlation between chlorophyll (CHL), total phosphorus (TP), and total nitrogen (TN) concentrations in north-temperate lake waters from around the world (Sakamoto 1966; Ahlgren 1980; Aizaki et al. 1981) and in Florida lakes (Huber et al. 1982; Canfield 1983). Large- and small-scale experiments further suggested that P was the primary limiting nutrient in northern lakes (Schindler 1975). Consequently, simple empirical TP–CHL regression models (Dillon and Rigler 1974; Jones and Bachmann 1976) have been used to predict changes in CHL concentrations (algal biomass) as a result of changes in TP concentrations.

In Florida, lake surveys using limited sampling (i.e., three times per year) have indicated a significant amount of variance in the yield of CHL per unit of TP (Huber et al. 1982; Canfield 1983). Several empirical nutrient–CHL models derived from this data yielded lower estimates of CHL than temperate models (Baker et al. 1981). Canfield (1983) further demonstrated that even the best empirical nutrient–CHL models had 95% confidence intervals of 29–319% of the calculated CHL concentration. He further suggested that this level of variance needs to be considered when using empirical models with TP and TN to predict CHL. Although TN seems to be an important factor influencing the yield of CHL per unit of TP (Smith 1982; Canfield 1983), studies of lakes suggest that other environmental factors such as sus-

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C.D. Brown,[2] M.V. Hoyer, R.W. Bachmann, and D.E. Canfield, Jr. Department of Fisheries and Aquatic Sciences, University of Florida, Gainesville, FL 32653, U.S.A.

[1]Journal Series No. R-07310 of the Florida Agricultural Experiment Station, Gainesville, FL 32653, U.S.A.
[2]Author to whom all correspondence should be addressed. e-mail: browncdb@ufl.edu

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pended solids (Hoyer and Jones 1983), aquatic plants (Canfield et al. 1984), lake flushing rates and light availability (Soballe and Kimmel 1987), and grazing effects (Shapiro 1979; Pace 1984) need to be considered. A number of these factors have led to reductions in the variance of predicted CHL concentrations when they were included in simple empirical models.

Some of the variability associated with published TP–CHL models has been attributed to the curvilinear nature of the general TP–CHL relationship with an asymptote around 100 μg TP·L^{-1} (Canfield 1983; Prairie et al. 1989). Straskraba (1980) suggested that the TP–CHL relationship is sigmoid and concluded that the slope coefficient of the TP–CHL relationship varies over a continuum of TP concentrations. McCauley et al. (1989) observed a sigmoid nature in the TP–CHL relationship for north-temperate lakes suggesting that a second nutrient such as TN had a significant impact on CHL values when TP concentrations were high. Mazumder and Havens (1998) described the TP–CHL relationship for both north-temperate lakes and Florida subtropical lakes as sigmoid. The apparent nonlinear character of the TP–CHL relationship based on mean CHL and TP concentrations has led researchers to revisit the assumption of limitation on which the relationship is based.

A recent paper (Kaiser et al. 1994) strongly emphasized the importance of the "law of the minimum" as a foundation of empirical nutrient–CHL models. Kaiser et al. (1994) also suggested that the description of maximum responses across levels of the potentially limiting factor (TP) should be the focus of statistical models derived from the law of the minimum and that all measured CHL concentrations below this line would indicate that environmental factors other than TP were limiting. A similar observation was advanced by Hosper (1980), who suggested that it would be more appropriate to interpret the effects of nutrients as posing an upper limit to summer CHL concentrations.

Over the last 10 years, a unique data set consisting of a large number of paired monthly nutrient and CHL estimates has been established by Florida LAKEWATCH, a citizen-based volunteer lake monitoring program (Florida LAKEWATCH 1998). The database represents paired sampling of monthly TP, TN, and CHL concentrations from 1986 to 1997, yielding over 12 450 monthly estimates from 360 lakes. All samples were collected following the same field procedures and analyzed in the same laboratory. This database permits a detailed examination of nutrient–CHL relationships.

In this paper, we use the Florida LAKEWATCH database to specifically examine TP–CHL relationships for Florida lakes using 1068 lake–year averages from 360 lakes. We further compare TP–CHL relationships in Florida with those of more northern lakes and evaluate the potential effects of TN on CHL yields because TN has been shown to be a limiting nutrient in some lakes (Smith 1982; Canfield 1983). We also evaluate the uncertainty associated with newly developed and previously published empirical nutrient–CHL models and establish a line expressing the maximum CHL response per unit of TP. Finally, we describe an empirical median line that describes the median CHL response per unit of TP for lakes having TP concentrations ranging from <1.0 to 100 μg·L^{-1}.

Materials and methods

Sampling program

Monthly TP, TN, and CHL data collected by citizen volunteers in the Florida LAKEWATCH program were used to establish a database to examine nutrient–CHL relationships. Canfield (1991) reported that water samples collected by citizen volunteers provided equivalent estimates of TP, TN, and CHL concentrations as data collected by professional biologists for 125 individual lakes.

In the field, citizen volunteers collected surface (0.5 m) water samples for TP and TN analyses from one to six evenly distributed locations. Water samples were collected using acid-washed, triple-rinsed 250-mL Nalgene bottles. Volunteers collected additional surface water samples at each location in tap water rinsed 4-L plastic bottles for CHL analyses. Upon returning to shore, a measured volume of lake water from the 4-L bottles was filtered through a Gelman type A-E glass fiber filter. All samples (water and filters) were frozen and sent to the Department of Fisheries and Aquatic Sciences' water quality laboratory where samples were analyzed for TP, TN, and CHL concentrations.

TP concentrations (micrograms per litre) were determined by the procedures of Murphy and Riley (1962) with a persulfate digestion (Menzel and Corwin 1965). TN concentrations (micrograms per litre) were determined by oxidizing water samples with persulfate and determining nitrate-N with second-derivative spectroscopy (Bachmann and Canfield 1996). CHL concentrations (micrograms per litre) were determined spectrophotometrically following pigment extraction with 90% ethanol (Sartory and Grobbelaar 1984).

Statistical procedures

A database consisting of paired annual mean nutrient and CHL data was established from the Florida LAKEWATCH database. Data from 360 lakes were collected on 11–13 dates over an annual cycle (January–December) for a minimum of 1 year. All stations within a lake (one to six) were averaged by month to obtain monthly means. For these 360 lakes, the data for each given year were designated as a lake–year average. This resulted in 1068 lake–year averages.

All analyses used in this paper were also conducted on data rearranged to avoid overweighting individual lakes by taking lake averages and by randomly selecting a single year for each lake with multiple years of data. The results were no different from the 1068 lake–year averages, so the results of the larger data set were reported.

Our 1068 lake–year averages data set was randomly sorted into two equal-sized data subsets to evaluate nutrient–CHL relationships for Florida lakes. A model development subset consisting of 533 lake–year averages from 274 lakes was used to develop three new empirical nutrient–CHL regression models using TP, TN, and TP + TN as variables in the models. A model confirmation subset consisting of 533 lake–year averages from 282 lakes was used to evaluate the predictive abilities of the three newly developed models and 10 published nutrient–CHL models. Models selected from the literature for comparison represent well-known work based on data covering a wide range of geographical areas and limnological conditions.

Four measures of precision (correlation coefficients, confidence limits, average errors, and percentage errors) were calculated for each new and published model following the procedures of Canfield (1983). Calculated CHL concentrations were obtained from developed and published nutrient–CHL models by using the measured TP and TN concentrations in the model confirmation subset. Pearson's correlation coefficients were determined for the relationship between measured and calculated CHL concentrations. An empirical 95% confidence limit (CL) was determined for the calculated CHL concentrations of each model by calculating the stan-

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dard deviation of the mean difference between the logarithms of the measured and calculated CHL concentrations. Standard deviations rather than standard errors were used because we were interested in the precision of estimating individual values rather than means. The standard deviation was multiplied by 1.96, the z value corresponding to a 95% CL given $n > 100$ (Dixon and Massey 1969). This value (standard deviation × 1.96) was both subtracted and added to the mean difference. Antilogs of the mean difference ± (standard deviation × 1.96) were multiplied by 100 to express values as a percentage. The average error was calculated as the mean of the absolute values of the differences between untransformed measured and calculated monthly CHL values. The percentage error was the mean of the same differences divided by the measured values and multiplied by 100.

A model development subset consisting of 533 seasonal means (average of July–August) from 274 lakes was used to develop a summer TP–CHL regression model for Florida. A model confirmation subset consisting of 533 seasonal averages from 282 lakes was used to evaluate the predictive abilities of the newly developed model and the Jones and Bachmann (1976) empirical model developed from 189 north-temperate lakes. The four measures of precision (correlation coefficients, confidence limits, average errors, and percentage errors) were then calculated for both models. In addition, $\log_{10}$-transformed seasonal measured TP concentrations from our database were used in the Jones and Bachmann (1976) equation to obtain predicted seasonal (calculated) $\log_{10}$ CHL concentrations. The antilog of the predicted CHL was subtracted from observed CHL and plotted versus TP concentrations to determine if the Jones and Bachmann (1976) equation predicted significantly different CHL concentrations for Florida lakes.

A maximum CHL line was determined using associated monthly TP and CHL data (n = 12 463 from Florida LAKEWATCH 1998). The monthly values for $\log_{10}$ TP and $\log_{10}$ CHL were sorted into 28 groups according to the values for $\log_{10}$ TP. The first group ranged from 0 to 0.1, the next from 0.1 to 0.2, and the last group from 2.7 to 2.8. We calculated the mean values for $\log_{10}$ TP and $\log_{10}$ CHL for each group and plotted them. We fitted a third-degree polynomial equation to the resulting sigmoid curve. This equation represents a mean P–CHL relationship that is equally weighted across the range of TP concentrations in our sample and was used to calculate an expected CHL concentration for each TP concentration. The differences between the actual and the calculated CHL concentration were found. The next step involved ranking all of the monthly values according to TP concentration and then taking the first 623 pairs and placing them in the first group, the next 623 pairs in the next group, and so on to form 20 groups. Within each group, the six CHL values that had the largest positive deviation above the line were plotted against their respective TP concentrations and then the next six highest points were plotted on the same graph. A smooth line was drawn roughly between these two sets of points to represent the most probable maximum P–CHL relationship. A polynomial regression was fitted through a set of points on that line.

A composite of 928 associated CHL and TP data from 818 lakes taken from the literature (e.g., Jones and Bachmann 1976; Aizaki et al. 1981; Prepas and Trew 1983) was used to create a representative global data set. Literature data were examined to avoid repeating data that were included in more than one study. The global data set and 1068 lake–year averages from 360 Florida lakes (Florida LAKEWATCH 1998) were used to evaluate differences between linear and sigmoid regression lines.

A linear regression line fitted to the 1068 lake–year averages was compared with a sigmoid curve developed from Florida monthly data. The same sigmoid curve was then compared with a linear regression line fitted to the global data set. Lakes in the global data set with TP concentrations less than 100 $\mu g \cdot L^{-1}$ were then used to develop a world median line to describe the TP–CHL relationship.

All data were transformed to their logarithms (base 10) before any statistical analyses to accommodate heterogeneity of variance. Computations were performed using various procedures in JMP (SAS Institute Inc. 1994). Statements of statistical significance are at $p \leq 0.05$ unless otherwise stated.

Results and discussion

The 360 Florida lakes used in this study represent a wide range of limnological conditions (Table 1). Mean TP concentrations ranged from 2.6 to 362 $\mu g \cdot L^{-1}$ and mean TN concentrations ranged from 56 to 4120 $\mu g \cdot L^{-1}$. Mean CHL concentrations ranged from <1.0 to 260 $\mu g \cdot L^{-1}$. The following additional summary statistics, although not available for all lakes, provide background information as to the types of lakes used to evaluate models. Surface areas ranged from 0.8 to 11 207 ha and average mean depths ranged from 0.4 to 7.7 m. Mean pH ranged from 4.1 to 9.8 and mean total alkalinity ranged from 0 to 137 $mg \cdot L^{-1}$ as calcium carbonate. Mean specific conductance ranged from 16 to 3050 $\mu S \cdot cm^{-1}$ and color averaged from <1.0 to 690 platinum–cobalt units. Mean water transparency ranged from 0.2 to 7.7 m. These ranges are similar to those previously reported by Canfield and Hoyer (1988) for a large data set of 165 Florida lakes. However, there are relationships despite the wide range of limnological conditions between CHL and nutrients (TP or TN) whether monthly or annual data are examined (Fig. 1).

Variance component analysis (SAS 1994) was used to determine those factors that contribute most to observed variability in measurements of TP, TN, and CHL. Variance component analysis indicated that 87, 86, and 66%, respectively, of the observed variance was attributable to differences among lakes. Less than 1% of the TP variance, 3% of the TN variance, and 15% of the CHL variance was associated with the year of sampling. Similar to Canfield (1983), the greatest amount of variance in measurements of TP, TN, and CHL in our data set is attributed to lake differences.

TP–CHL relationships

A plot of associated monthly mean CHL as a function of monthly mean TP suggested a sigmoid pattern for the TP–CHL relationship in Florida lakes. A third-order polynomial regression for mean CHL response was developed to evaluate the sigmoid pattern in terms of best fit for the data cluster. The curve fitted to the data is described by the following equation:

$$(1) \quad \log(\mathrm{CHL}) = 0.078 - 0.42\log(\mathrm{TP}) + 1.27\log(\mathrm{TP})^2 - 0.32\log(\mathrm{TP})^3$$

where CHL and TP are the average of one to six stations sampled once monthly. The p value for each coefficient in the polynomial equation was statistically significant. The predicted CHL responses of the polynomial equation (R^2 = 0.69) and a simple least squares regression line (R^2 = 0.67) fitted to the same data, however, were highly correlated (r = 0.99). Although the polynomial curve explains more variance of CHL estimates than the linear regression equation, the strong correlation for predicted CHL estimates suggests that both models would not predict very different mean CHL

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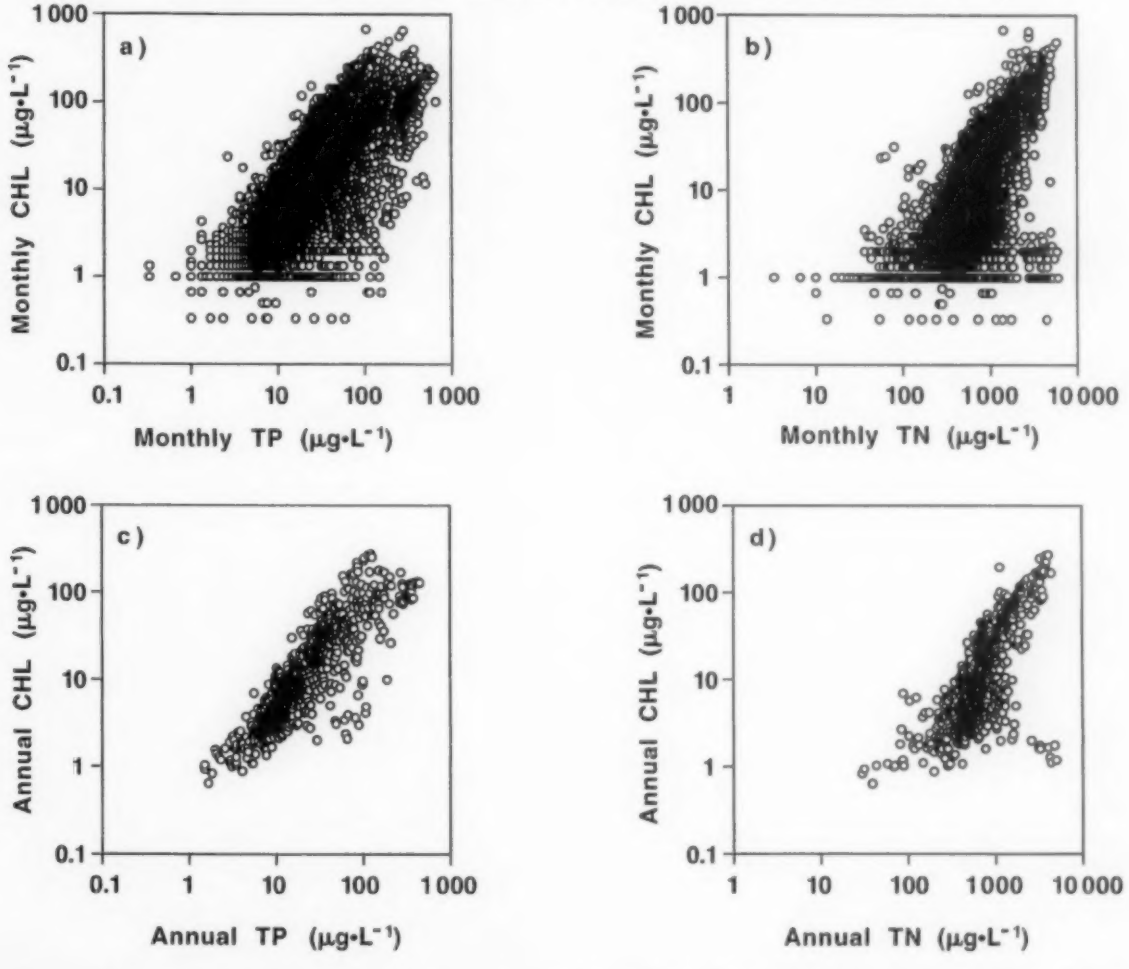
Table 1. Summary statistics of available average limnological data from 360 Florida lakes located in 29 counties.

	n	Minimum	Median	Mean	Maximum
Surface area (ha)	262	0.8	50	360	11 207
Mean depth (m)	199	0.4	2.7	3.1	7.7
pH	231	4.1	7.0	6.9	9.8
Total alkalinity ($mg \cdot L^{-1}$ as $CaCO_3$)	231	0	13	24	137
Specific conductance ($\mu S \cdot cm^{-1}$ at 25°C)	231	16	141	167	3 050
Color (Pt–Co units)	231	<1.0	20	44	690
TP ($\mu g \cdot L^{-1}$)	360	2.6	18	37	362
TN ($\mu g \cdot L^{-1}$)	360	56	690	860	4 120
TN/TP ratio	360	5	35	42	1 157
CHL ($\mu g \cdot L^{-1}$)	359	<1.0	8.9	23	260
Secchi (m)	354	0.2	1.7	1.9	7.7

Note: Lakes were sampled monthly by Florida LAKEWATCH citizen volunteers from 1986 through 1997 for estimates of TP, TN, and CHL concentrations.

Fig. 1. TP–CHL and TN–CHL relationships for the association of 12 463 months and 1068 annual Florida lake averages.



responses. The same results were observed for a third-order polynomial ($R^2 = 0.78$) and a linear equation ($R^2 = 0.76$) fitted to 1068 Florida lake–year averages of TP and CHL. The similarity of the slopes for both equations over a wide range of TP from log 0.5 (3 $\mu g \cdot L^{-1}$) to log 2.2 (160 $\mu g \cdot L^{-1}$) indicates that simple models provide similar mean CHL esti-

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mates, particularly for TP concentrations, which would be considered most limiting. More than 93% of mean TP values from Florida and most published data sets fall in this range ($\leq$160 $\mu g \cdot L^{-1}$).

It has been shown that three or four samples collected over a year can be used to estimate mean annual CHL concentrations in Florida lakes with a 25–35% coefficient of variation (Brown 1997). Earlier models describing the TP–CHL relationship in Florida were developed using lake survey data with this level of variance associated with sampling design. Using monthly data to obtain annual estimates of CHL concentrations results in a 15% coefficient of variation (Brown 1997); however, there remains considerable variation in the amount of CHL yielded per unit of TP (0.03–2.6) or per unit of TN (<0.001–0.17) using annual data based on monthly sampling (Fig. 1).

New empirical Florida nutrient–CHL models based on annual CHL and nutrient averages

Empirical nutrient–CHL models were developed from the model development subset using the annual means based on monthly data for Florida lakes (n = 533 lake–year averages from 274 lakes) (Table 2). TP alone accounted for a significant amount of the variance (R^2 = 0.76) of observed CHL measurements (Table 2). TN alone accounted for less variance of observed CHL measurements (R^2 = 0.46), but a multivariate model using both TP and TN also accounted for a significant amount of the observed variance (R^2 = 0.78). The coefficient of determination values for TP–CHL and the multivariate nutrient–CHL model, however, were similar (R^2 = 0.76 versus 0.78) (Table 2), suggesting that CHL concentrations can be predicted reasonably well using TP alone.

Precision of empirical TP–CHL models

The model confirmation subset consisting of 533 lake–year averages from 282 lakes was used to test the abilities of the newly developed empirical models and 11 published nutrient–CHL models to predict CHL values (Tables 3, 4, and 5). The new TP–CHL model developed from annual Florida lake data (Table 3) had the smallest 95% CL (30–325% of the calculated CHL value) and average error (13%). Even assuming an 80% CL, CHL estimates ranged from 46 to 216% of the calculated CHL value.

Of the published Florida models, the Canfield (1983) model had the second lowest average error (15%) and smallest percentage error (49%) and the Huber et al. (1982) model had the highest average error (30%) and percentage error (84%). Even though all of the TP–CHL models had similar correlation coefficients (r = 0.87) for measured versus calculated CHL values, there remains a large degree of uncertainty in CHL predictions using TP alone (Table 3).

July–August TP–CHL relationships: Florida lakes versus northern lakes

The mean TP and CHL concentrations in Florida lakes during July–August ranged considerably as did July–August concentrations for northern lakes, as reported by Jones and Bachmann (1976). Average TP ranged from 1.0 to 390 $\mu g \cdot L^{-1}$ for Florida lakes and TP ranged from 2.7 to 350 $\mu g \cdot L^{-1}$ for northern lakes. Mean CHL ranged from <1.0 to 315 $\mu g \cdot L^{-1}$ for Florida lakes and CHL ranged from <1.0 to 400 $\mu g \cdot L^{-1}$ for northern lakes. The range of observed CHL per unit of TP (0.02–3.4) for Florida lakes was greater than the range (0.1–2.1) observed for northern lakes, suggesting that some Florida lakes during July and August may have more CHL per unit of TP than northern lakes.

A Florida TP–CHL model developed from 533 mean measured TP and CHL values collected in July and August from 273 lakes was somewhat different from the Jones and Bachmann (1976) north-temperate TP–CHL model (Table 4). The slope for the Florida model (m = 1.03) was not significantly different from 1.0. The north-temperate model's slope (m = 1.46) was significantly greater than 1.0, and the model's intercept (b = –1.09) was less than the Florida model's intercept (b = –0.299), indicating that the two lines would intersect at a TP concentration of around 63 $\mu g \cdot L^{-1}$. The northern equation would therefore tend to predict higher CHL concentrations at high TP concentrations and lower CHL concentrations at low TP concentrations. The coefficient of determination for the relationship between TP and CHL for both equations was positive and significant. The coefficient of determination of the Florida data set (R^2 = 0.72) was lower compared with that of the Jones and Bachmann (1976) data set (R^2 = 0.90).

Both the Florida and northern seasonal models were tested for their ability to predict CHL values in a July–August model confirmation subset (Table 4). The Florida model had lower average error (14%) and percentage error (65%) and smaller 95% CL (27–365% of the calculated CHL value) compared with the Jones and Bachmann (1976) model. Both TP–CHL equations had similar correlation coefficients (r = 0.86) for measured and calculated CHL values. The Jones and Bachmann (1976) TP–CHL model was less precise when used to predict seasonal (July–August mean) CHL concentrations for Florida.

Jones and Bachmann (1976) used July–August averages of TP because P was considered limiting during this time of year, with most of the TP in the water column involved in the algal population. In north-temperate regions, distinct seasonal cycles of algal biomass are correlated with temperature and solar radiation patterns that are considered to limit algal biomass at other times of the year. In Florida, there is evidence that the maximum CHL per unit of TP is not limited to July–August and could occur at other times of the year (Brown et al. 1998). The Jones and Bachmann (1976) model underestimates CHL concentrations in Florida lakes at extremely low TP concentrations and overestimates CHL concentrations at extremely high TP concentrations (Fig. 2). Reasonable estimates of CHL, however, were obtained for the majority of Florida lakes. Models derived from the basic TP–CHL relationships for July–August Florida and north-temperate lake data sets also share a large number of common data points, with the majority of these data points falling in a large cluster within certain confidence limits. Slope differences between the two models tend to mask similarities in the fundamental TP–CHL relationships (Fig. 2; Table 4).

TN–CHL relationships

A plot of associated monthly mean CHL as a function of monthly mean TN also suggested a sigmoid pattern for the TN–CHL relationship in Florida lakes. A third-order polynomial regression for mean CHL response was developed to

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Table 2. Three new empirical models and associated statistics.

Models	n	F	$p > F$	R^2
log(CHL) = –0.369 + 1.053log(TP)	533	1712	<0.01	0.76
log(CHL) = –2.42 + 1.206log(TN)	533	444	<0.01	0.46
log(CHL) = –1.10 + 0.91log(TP) + 0.321log(TN)	533	949	<0.01	0.78

Note: The models describe the association of annual average nutrient and CHL concentrations collected monthly at the same time and location for 273 Florida lakes (Florida LAKEWATCH 1998).

Table 3. Correlation (r) between measured and calculated CHL concentrations for TP–CHL models evaluated using the model confirmation subset.

		Error estimates		
Model	r	AE	PE	CL
Florida models				
Florida LAKEWATCH (1998), annual				
log(CHL) = –0.369 + 1.053log(TP)	0.87	13	58	30–325
Baker et al. (1981)				
log(CHL) = –0.41 + 0.79log(TP)	0.87	18	57	66–900
Huber et al. (1982)				
log(CHL) = –1.08 + 1.52log(TP)	0.87	30	84	27–553
Canfield (1983)				
log(CHL) = –0.15 + 0.744log(TP)	0.87	15	49	40–593
Northern models				
Dillon and Rigler (1974)				
log(CHL) = –1.14 + 1.449log(TP)	0.87	21	70	41–725
Jones and Bachmann (1976)				
log(CHL) = –1.09 + 1.46log(TP)	0.87	23	72	35–633
Midwestern model				
Hoyer (1981)				
log(CHL) = –0.77 + 1.24log(TP)	0.87	16	56	40–488

Note: Error estimates include average error (AE), percentage error (PE), and 95% CL as percentages of the calculated CHL values.

Table 4. Correlation (r) between measured and calculated CHL concentrations for TP–CHL models evaluated using the model confirmation subset.

		Error estimates		
Model	r	AE	PE	CL
Florida LAKEWATCH (1998), July–August				
log(CHL) = –0.299 + 1.03log(TP)	0.86	14	65	27–365
Jones and Bachmann (1976)				
log(CHL) = –1.09 + 1.46log(TP)	0.86	25	77	36–771

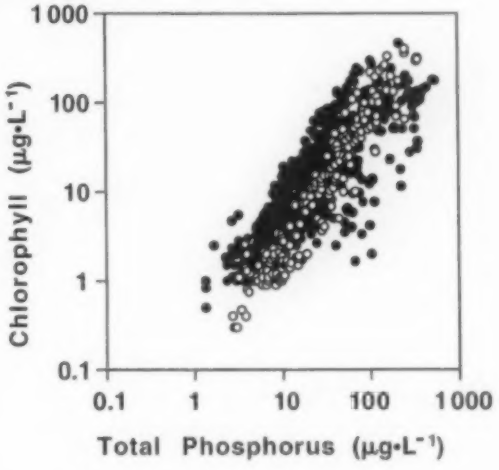
Note: Error estimates include average error (AE), percentage error (PE), and 95% CL as percentages of the calculated CHL values. Seasonal confirmation tests of the Florida LAKEWATCH (1998) and Jones and Bachmann (1976) equations used average July–August data from the model confirmation subset (n = 282 lakes).

evaluate the sigmoid pattern in terms of best fit for the data cluster. The curve fitted to the data is described by the following equation:

$$(2) \quad \log(CHL) = 5.35 - 7.14\log(TN) + 2.87\log(TN)^2 - 0.32\log(TN)^3$$

where CHL and TN are the average of one to six stations sampled once monthly. The p value for each coefficient in the polynomial equation was statistically significant. The predicted CHL responses of the polynomial equation (R^2 = 0.50) and a simple least squares regression line (R^2 = 0.48) fitted to the same data, however, were highly correlated (r = 0.97) for the entire range of TN concentrations. Similar results were noted for a polynomial (R^2 = 0.57) and linear equation (R^2 = 0.54) fitted to 1068 Florida lake–year averages of associated TN and CHL concentrations. The slopes for both equations were similar for a wide range of TN from log 2.1 (126 $\mu g \cdot L^{-1}$) to log 3.8 (6300 $\mu g \cdot L^{-1}$), suggesting that they provide comparable mean CHL estimates.

Fig. 2. Comparison of average July–August data used to derive Jones and Bachmann (1976) north-temperate model (n = 189) and a new Florida model (n = 533) derived from annual averages based on monthly sampling. The Jones and Bachmann (1976) data are represented as open circles and the Florida data as solid circles.



Precision of empirical TN–CHL and (TP + TN)–CHL models

Examining TN–CHL models shows that the new TN–CHL model developed from annual Florida lake data collected monthly had the smallest 95% CLs (23–486% of the calculated CHL value) of all TN–CHL models evaluated (Table 5). The average error of 14% was also the smallest of all TN–CHL models examined. Comparison of percentage error estimates calculated for each TP–CHL model with percentage error estimates for each TN–CHL model showed that TP models had greater precision in estimating CHL concentrations in Florida lakes (Tables 3 and 5).

Comparing simple TP–CHL relationships with multivariate (TP + TN) models indicates that percentage errors were similar (Table 5). Increases in precision were observed, how-

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Table 5. Correlation (r) between measured and calculated CHL concentrations for TN–CHL and multivariate nutrient–CHL models.

Model	r	Error estimates AE	PE	CL
Florida TN–CHL models				
Florida LAKEWATCH (1998)				
log(CHL) = –2.42+ 1.206log(TN)	0.79	14	76	23–486
Canfield (1983)				
log(CHL) = –2.99 + 1.38log(TN)	0.79	14	66	29–562
Midwestern TN–CHL model				
Hoyer (1981)				
log(CHL) = –1.23 + 0.798log(TN)	0.79	16	90	18–552
Florida multivariate nutrient–CHL models				
Florida LAKEWATCH (1998), all data				
log(CHL) = –1.10 + 0.91log(TP) + 0.321log(TN)	0.88	12	55	33–311
Smith (1982)				
log(CHL) = –2.488 + 0.374log(TP) + 0.935log(TN)	0.87	17	57	68–796
Canfield (1983)				
log(CHL) = –2.49 + 0.269log(TP) + 1.06log(TN)	0.85	14	54	39–518
Midwestern multivariate nutrient–CHL model				
Hoyer (1981)				
log(CHL) = –1.136 + 1.19log(TP) + 0.155log(TN)	0.88	16	56	40–473

Note: All models were evaluated using the model confirmation subset. Error estimates include average error (AE), percentage error (PE), and 95% CL as percentages of the calculated CHL values. See text for methods of determination.

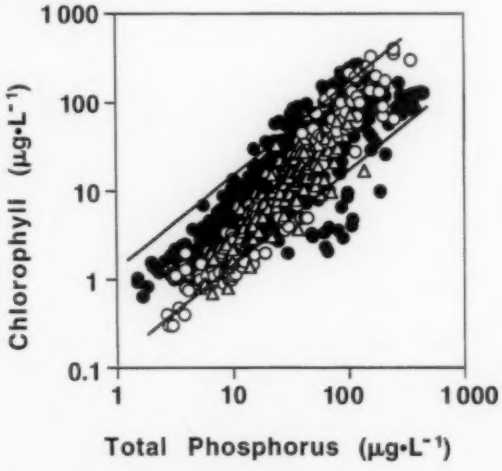
ever, with the addition of TN to simple TP–CHL models (Table 5). While the addition of TN to published TP–CHL models led to reductions in variance for Florida and northern lakes (Smith 1982; Canfield 1983), the 95% CLs for all of the empirical models examined remained large (33–311% to 68–796%).

Commonality of data associations

Researchers have noted a bending in simple TP–CHL models at high concentrations of TP (Forsberg and Ryding 1980; Canfield and Bachmann 1981). Canfield (1983) observed the tendency of published empirical TP–CHL models to overestimate CHL values in lakes with high TP concentrations (>100 $\mu g \cdot L^{-1}$). Some earlier Florida empirical nutrient–CHL models predict lower estimates of CHL per unit of TP than temperate models (Baker et al. 1981). Inspection of Florida July–August averages indicated that Florida lakes with seasonal CHL concentrations < 100 $\mu g\ TP \cdot L^{-1}$ do not necessarily have lower algal CHL per measured TP concentration when compared with north-temperate lakes (Fig. 2).

Once again, we reiterate that all data sets examined here have a large number of common data points. Enumeration of the plotted data points that fall above or below the confidence limits associated with some published models (Jones and Bachmann 1976; Hoyer and Jones 1983) indicated that 5–18% of the Florida, northern, or midwestern data points fell outside any other model's 95% predictive CLs. This suggests, as noted by Kaiser et al. (1994), that TP–CHL models describe the same basic TP–CHL relationship (Fig. 3). The large number of common data points shared among different data sets also confirms the Kaiser et al. (1994) supposition of an upper CHL response per unit of TP. Consequently, a maximum line of CHL response per unit of TP could be used as a benchmark to evaluate other limiting factors.

Fig. 3. Linear regression lines from several published models fall within the 95% CLs of the 1998 Florida LAKEWATCH database (n = 12 463) monthly TP and CHL associations. Temperate lake data of Jones and Bachmann (1976) are represented by open circles and of Hoyer (1981) by triangles, and Florida lake data are represented by solid circles.



Monthly data provide the greatest amount of information concerning the annual range of possible CHL values along a continuum of TP values (Brown 1997). In an attempt to address the maximum CHL response in terms of the nonlinear nature of the TP–CHL relationship, a sigmoid maximum CHL response curve was generated from 12 463 months of paired TP and CHL values from Florida lakes (Fig. 4). The maximum CHL response curve is described by the following equation:

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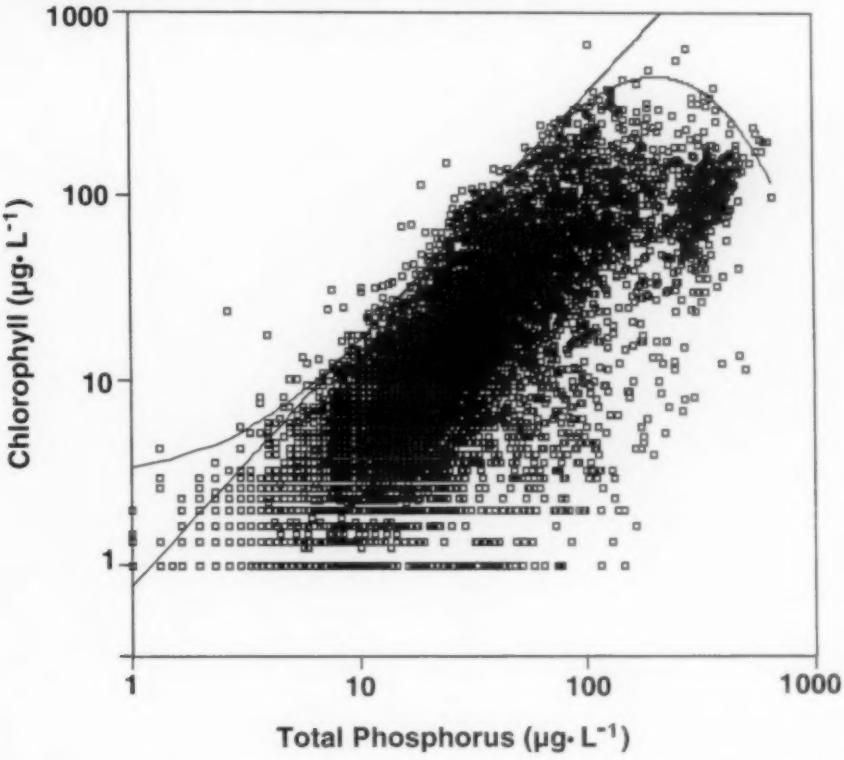
Fig. 4. Comparison of a calculated sigmoid and linear maximum CHL response curves generated from 12 463 months of associated TP and CHL concentrations from 360 Florida lakes in 29 counties from 1986 to 1997.



(3) $\log(\text{maximum CHL}) = 0.528 + 0.18\log(\text{TP}) + 0.33\log(\text{TP})^2 + 0.29\log(\text{TP})^3 - 0.128\log(\text{TP})^4$

where maximum CHL and TP are the average of one to six stations sampled once monthly. We interpret the maximum CHL response curve as describing P limitation (nutrient limitation) when the CHL response falls on or near the line and indicating other limiting environmental factors when the CHL response falls below the line. For example, Florida lakes that fall below the line with low TP are generally dark-color lakes. Other lakes that fall below the line with high TP generally have low TN/TP ratios, suggesting that TN may be limiting rather than TP (Fig. 4). The maximum CHL response curve is essentially linear over a range of TP concentrations from 8 to 76 $\mu g \cdot L^{-1}$ and can also be estimated by the following equation:

(4) $\log(\text{maximum CHL}) = -0.12 + 1.33\log(\text{TP})$

where maximum CHL and TP are the average of one to six stations sampled once monthly. A multiple analysis of variance of the predictive abilities of both the curvilinear and linear maximum CHL response models over this range is identical. When we expand the range to include TP concentrations from 3 to 100 $\mu g \cdot L^{-1}$, the predicted CHL responses are still highly correlated ($r = 0.99$). Only when we begin to include CHL responses for TP concentrations >100 $\mu g \cdot L^{-1}$ does the characteristic bending of the sigmoid relationship significantly reduce expected maximum CHL responses.

As stated above, lakes with high TP tend to have low TN/TP ratios and are considered N limited (Forsberg and Ryding 1980; Canfield 1983). There is evidence that CHL levels associated with TP concentrations above 100 $\mu g \cdot L^{-1}$ are influenced more by TN (Fig. 4). In the Florida LAKEWATCH database, 32 lakes with 90 lake–year averages of data have high TP concentrations (>100 $\mu g \cdot L^{-1}$). Simple univariate models derived from this data range indicate that TN is more strongly correlated ($R^2 = 0.55$) than TP ($R^2 = 0.05$) with CHL. Also, the TP coefficient is no longer significant in a multivariate nutrient–CHL model developed from the same data. The median TN/TP ratio for this data is 10 compared with 38 for lakes with lower TP concentrations (≤100 $\mu g \cdot L^{-1}$). Canfield (1983) observed a bending in the Florida TP–CHL relationship similar to that for lakes in other areas (Forsberg and Ryding 1980; Canfield and Bachmann 1981) where it appears that lakes with TP concentrations above 100 $\mu g \cdot L^{-1}$ are not limited by TP. These observations support the contention that TP is no longer limiting above 100 $\mu g \cdot L^{-1}$ and that N takes on a more important role.

Cursory observation of monthly Florida lake data suggested that factors other than TP may also limit CHL responses in a large number of lakes at concentrations below 100 $\mu g \cdot L^{-1}$ (Fig. 3). Environmental factors such as suspended solids (Canfield and Bachmann 1981; Hoyer and Jones 1983), color, and low TN/TP ratios have been shown to influence CHL responses in other areas and would also

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tend to cause CHL responses in Florida to fall below the maximum line. Suspended solids and color can lead to light attenuation that would potentially reduce CHL responses at any TP concentration.

A straight line fitted to the global data set and plotted with the sigmoid curve of means based on Florida monthly data indicated that Florida mean CHL responses fall above the straight-line regression of TP–CHL for the global data set over a wide range of TP from log 1.0 (10 $\mu g \cdot L^{-1}$) to log 2.4 (250 $\mu g \cdot L^{-1}$). When a polynomial equation was fitted to the global data set, the lower end of the sigmoid tail was no longer evident and the curve fell entirely below the sigmoid curve of Florida means. Overall, Florida lakes do not have less CHL than lakes in the global data set.

Management alternative: the global median

There are a number of empirical models that are reported to be different from each other. These differences are most likely the result of the range of data incorporated in the databases. Florida annual data from 1068 lake–years were combined with the global data set to expand the range of possible CHL responses. Other empirical models used to predict CHL response per unit of TP often incorporate other known factors when available to explain more of the variance associated with CHL estimations. In some instances, other factors remain unknown, so a general model based on median mean CHL response could provide reasonable estimates of CHL responses: 50% of responses would be greater and 50% would be less than the observed concentration. Such a model can be used for ballpark CHL estimates in an individual lake as well as in a population of lakes without the drawback of trying to find a specific model to apply to individual lake cases or develop models for each individual lake.

To assure the generality of a median model for universal application requires it to be derived from a large population of lakes from around the globe. We calculated a global median line using paired mean TP and CHL data from lakes in Europe, Japan, and the United States with TP concentrations <100 $\mu g \cdot L^{-1}$. The global median line is described by the following equation:

$$(5) \quad \log(\text{median CHL}) = -0.44 + 1.10\log(\text{TP})$$

where median CHL and TP are the average of one to six stations sampled once monthly. This line predicts the median expected mean CHL concentration. Lakes with TP concentrations >100 $\mu g \cdot L^{-1}$ were excluded when determining the median line in an attempt to reduce the possible influence of N-limited lakes. As associated TN data were not available for the complete global data series, it was impossible to sort on the basis of the TN/TP ratio.

The global median line can be a useful tool to predict CHL yields, as it maintains the generality of the TP–CHL relationship and was derived from data collected from a broad range of lakes. The global median is adequate for general application, but there are published models that may be more appropriate if certain colimiting factors are known to affect CHL yields in lakes within a region. For example, in Missouri reservoirs where suspended solids may bind nutrients or reduce light, Hoyer and Jones (1983) found that incorporating suspended solids into their TP–CHL model explained more CHL variance.

Summary and conclusion

In this study, Florida models were reconstructed and tested on annual means derived from monthly data. However, there is still a limit to the precision of the simple nutrient–CHL models. The 95% CL for the best available TP–CHL model is 30–325% of the calculated CHL value. Even assuming an 80% CL, CHL estimates ranged from 46 to 216% of the calculated CHL value. The best available multivariate model (TP + TN) has a 95% CL of 33–311% of the calculated CHL value. These large confidence intervals are not suprising, as similarly large intervals have previously been reported for nutrient loading models that predict P (31–288%; Canfield and Bachmann 1981) and N (41–255%; Bachmann 1980) concentrations.

Analyses of associated nutrient and CHL data indicate that the TP–CHL relationship is sigmoid in character but that the majority of CHL responses for TP concentrations (3–160 $\mu g \cdot L^{-1}$) are not much different from estimates from linear relationships for all practical purposes.

A large number of common data points shared among different data sets suggested that there is an upper response per unit of TP often representing algal bloom conditions. A maximum CHL response curve derived from over 12 400 months of paired TP and CHL data from Florida lakes is sigmoid. The maximum CHL response for both the curve and straight line is similar for a wide range of TP concentrations (3–100 $\mu g \cdot L^{-1}$). The maximum CHL response curve and straight line both define the upper limit of expected CHL concentrations based on the TP–CHL relationship and provide a benchmark to evaluate other limiting factors. The maximum curve describes P limitation when the CHL response falls on or near the line but indicates other limiting or colimiting factors when the CHL response falls below the line.

Florida lakes do not yield less CHL than north-temperate lakes. Both Florida and global data sets are similar, exhibiting a lessening of slope above a TP concentration of 100 $\mu g \cdot L^{-1}$. Almost all of the global data points fall below the maximum CHL response curve, as they are means and would in effect vary less than the monthly data used to develop the maximum CHL response.

The large confidence intervals associated with CHL predictions reflect the real-world variability of algal biomass in lakes as well as the limitation of simple mathematical descriptions of complex biological systems. Different responses of individual lakes to changes in TP account for a large amount of the total variance in TP–CHL regression models. Each lake's individual response confounds the general assumption that individual lakes will respond in a similar fashion to a given change in TP concentration. Efforts to describe the TP–CHL relationship using models more representative of the law of the minimum (Kaiser et al. 1994) are often complex, but the underlying concept remains important for applied lake management. Other factors beyond nutrients need to be considered, particularly when applying population models to individual lakes.

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Role of chemical and physical variables in regulating microcystin-LR concentration in phytoplankton of eutrophic lakes

Brian G. Kotak, Angeline K.-Y. Lam, Ellie E. Prepas, and Steve E. Hrudey

Abstract: A survey of eutrophic to hypereutrophic hardwater lakes in central Alberta was conducted to test the hypotheses that the concentration of the cyanobacterial toxin microcystin-LR (MC-LR) in phytoplankton is regulated by environmental factors that affect both the biomass of the main producer of the toxin, *Microcystis aeruginosa*, and the concentration of the toxin in the cells. Of all environmental factors examined, total phosphorus was the strongest correlate of both *M. aeruginosa* biomass and cellular MC-LR (expressed as micrograms per gram of *M. aeruginosa*). *Microcystis aeruginosa* biomass was also strongly negatively related to the total nitrogen to total phosphorus ratio (TN:TP) and inorganic nitrogen (NO_2^- + NO_3^-, NH_4^+). A univariate regression model of TN:TP explained the most variation in MC-LR concentration (expressed as nanograms of cellular toxin per litre) in mixed phytoplankton communities. This study indicated that MC-LR dynamics in phytoplankton of lakes was related to changes in the concentration and ratio of phosphorus and nitrogen.

Résumé : Nous avons effectué une étude sur des lacs eutrophes à hypereutrophes aux eaux dures du centre de l'Alberta pour vérifier l'hypothèse selon laquelle la concentration de la toxine cyanobactérienne microcystine-LR (MC-LR) dans le phytoplancton serait régulée par des facteurs environnementaux qui touchent à la fois la biomasse du producteur principal de la toxine, *Microcystis aeruginosa*, et la concentration de la toxine dans les cellules. Parmi tous les facteurs environnementaux examinés, c'est le phosphore total qui présentait la plus forte corrélation avec à la fois la biomasse de *M. aeruginosa* et la concentration cellulaire de MC-LR (exprimée en $\mu g \cdot g^{-1}$ *M. aeruginosa*). La biomasse de *M. aeruginosa* était aussi fortement et négativement corrélée au rapport de l'azote total sur le phosphore total (NT:TP) et à l'azote inorganique (NO_2^- + NO_3^-, NH_4^+). Un modèle de régression unidimensionnelle de NT:PT expliquait la plus grande partie de la variation de la concentration de MC-LR (exprimée en ng de toxine cellulaire$\cdot L^{-1}$) dans les communautés phytoplanctoniques mixtes. L'étude indique que la dynamique de la MC-LR dans le phytoplancton des lacs était reliée à des changements dans la concentration et dans le rapport du phosphore et de l'azote.

[Traduit par la Rédaction]

Introduction

Cyanobacteria are commonly found in productive lakes worldwide. Their ubiquitous distribution and ability to dominate phytoplankton communities are related to a complex interaction among many factors. These factors include the concentration and ratio of nutrients such as phosphorus and nitrogen, the ability of certain genera to fix atmospheric nitrogen, buoyancy control and minimal grazing by zooplankton, among others (see review by Paerl 1996).

Several genera of cyanobacteria, including species of *Microcystis* (Kotak et al. 1993, 1995), *Anabaena* (Harada et al. 1991), *Oscillatoria* (Meriluoto et al. 1989), and *Nostoc* (Namikoshi et al. 1990), also produce potent liver toxins, termed microcystins, that have caused sporadic but repeated poisonings and deaths of animals worldwide (Schwimmer and Schwimmer 1968). Microcystins comprise a family of more than 50 closely related cyclic heptapeptides (Tyagi et al. 1999), many identified by substitution of amino acids on two positions of the structure. Microcystin-LR (MC-LR), named for two amino acids, leucine (L) and arganine (R), is the most commonly occurring microcystin analogue in central Alberta lakes (Kotak et al. 1993, 1995), one of the few regions worldwide where spatial and temporal patterns of toxin concentration have been explored. MC-LR is also one of the most toxic microcystins, with an LD_{50} of 50 $\mu g \cdot kg^{-1}$ in mice (Carmichael 1988). Microcystins pose a hazard to human health in that accidental consumption has led to an increased incidence of liver cancer (Fujiki and Suganuma 1993) as well as deaths of dialysis patients in South America (Dunn 1996).

The regulation of toxin production in cyanobacteria is not well understood. Several laboratory studies have examined the effects of various environmental factors (e.g., nutrients, light, temperature) on microcystin production in laboratory isolates of *Microcystis aeruginosa* (e.g., Van der Westhuizen and Eloff 1985; Watanabe and Oishi 1985; Utkilen and Gjolme 1992). Nutrients such as phosphorus and nitrogen

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B.G. Kotak,[1] A.K.-Y. Lam, and E.E. Prepas. Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada.
S.E. Hrudey. Environmental Health Program, Faculty of Medicine, University of Alberta, Edmonton, AB T6G 2G3, Canada.

[1]Author to whom all correspondence should be sent at the following address: AlgalTox International, P.O. Box 268, Pine Falls, MB R0E 1M0, Canada. e-mail: bkotak@granite.mb.ca

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appear to be key factors controlling toxin production. However, given that the saturating conditions, which are present in such laboratory studies, would rarely be expected to occur for most variables in the natural environment, the results from these laboratory studies need to be complemented by studies conducted in the field.

Examination of data collected from lakes presents an alternative method for gaining insights into the factors that may influence the production of cyanobacterial toxins in phytoplankton communities. The almost global occurrence of MC-LR in productive lakes makes this particular microcystin analogue a logical choice for such studies. There is a paucity of published studies relating toxin concentration in samples of mixed phytoplankton assemblages to environmental variables (Wicks and Theil 1990; Kotak et al. 1995). Further, Kotak et al. (1995) provided the only attempt where the relationships between MC-LR concentration in phytoplankton and environmental factors were quantified. Given the limited knowledge on the patterns of MC-LR concentration, and how these patterns relate to changes in environmental variables, data based on a larger suite of lakes and longer time series are needed to understand the factors that may influence toxin occurrence and concentrations in lakes.

In this paper, we test the hypotheses that MC-LR in phytoplankton communities is regulated through the influence of environmental factors (physical, chemical, and biological) on the abundance and biomass of the primary producer of the toxin (*M. aeruginosa*) and on toxin production in the cells. To do this, we surveyed 12 lakes (13 lake basins) in Alberta, Canada, from 1990 to 1994 in which the relationships between MC-LR concentration and environmental variables and cyanobacterial species were examined by correlation and regression analysis.

Materials and methods

Study lakes

All of the 13 lake basins are located in central and northern Alberta and are within the aspen parkland and boreal mixed-wood ecoregions or boreal plains. The study lakes are eutrophic to hypereutrophic (Mitchell and Prepas 1990). Animal poisonings that have been linked to cyanobacterial toxins have been reported in five of the lakes. Two of the lakes in this survey (Figure Eight and Halfmoon South lakes) have been treated on several occasions with lime (either as calcium hydroxide, calcium carbonate, or both) to reduce total phosphorus (TP) concentrations and algal biomass. Baptiste Lake has two basins (North and South) and each basin was sampled separately. The lakes were sampled approximately every 2 weeks during the open-water season, generally from May to October, although there were variations in annual sampling effort and chemical analyses, as some data were collected as part of other research projects.

Water collection and analysis

With the exception of Little Beaver Lake, in which water was collected as surface grabs in 1990 and from the surface to a depth of 1 m in the remaining years, water samples in all other lakes were collected at four sites per lake with Tygon tubing fitted with a one-way valve (samples represent integrated water from the lake surface to a depth of 2 m). Water samples collected from the four sites were combined prior to chemical analysis. In 1991, water samples were analysed for TP, $NO_3^- + NO_2^-$, phytoplankton biomass (chlorophyll *a* (Chl *a*)), conductivity, pH, and alkalinity on each sampling date. Other variables (Cl^-, SO_4^{2-}, Na^+, K^+, Ca^{2+}, Mg^{2+}, color, and turbidity) were analysed on water samples collected every second sampling date in 1991. In other years (i.e., 1990, 1992, 1993, and 1994), various limnological variables were not collected, thus decreasing the degrees of freedom for correlations based on the 1991 and the combined (1990–1994) data.

TP was analysed by colorimetric methods after potassium persulfate digestion (Menzel and Corwin 1965, as modified by Prepas and Rigler 1982), although large particles (>250 μm) were not filtered out. $NO_3^- + NO_2^-$ and NH_4^+ were determined by colorimetric methods on a Technicon Autoanalyzer II (Stainton et al. 1977), and phytoplankton biomass was estimated as Chl *a* after ethanol extraction (Bergmann and Peters 1980). Cl^- and SO_4^{2-} were analysed by ion chromatography on a Dionex Series 2000i/SP ion chromatograph and Ca^{2+}, K^+, Mg^{2+}, and Na^+ by atomic absorption on a Perkin-Elmer model 3300 atomic absorption unit, with methods described in APHA et al. (1992). In 1990 and 1991, total alkalinity was determined by manual titration of the water samples with 1×10^{-4} M sulfuric acid (APHA et al. 1992), and pH was measured on a Fisher Accumet model 925 pH meter. In 1992–1994, total alkalinity was determined on a Mettler DL21 autotitrator. Conductivity, color, and turbidity were measured on a Radiometer Analytical model CDM83 conductance meter, a Hellige AquaTester model 611-A meter, and a Hach model 2100A turbidity meter, respectively. In 1993, total nitrogen (TN) was determined as the sum of total Kjeldahl nitrogen (D'Elia et al. 1977) plus $NO_3^- + NO_2^-$. Water temperature was taken in situ at one central site in each lake with a YSI model 51 temperature/dissolved oxygen meter from the surface to the sediments at 1-m intervals. Mean water temperature from the depth of water collection (e.g., 0–2 or 0–1 m) was then calculated. Secchi depth was also recorded at one central site. Lake morphometry and watershed characteristics were obtained from Mitchell and Prepas (1990) and Kotak et al. (1993).

Phytoplankton were preserved in Lugol's solution from surface (0–0.5 m in 1990 and 1991 and 0–2 or 0–1 m in 1992–1994) water samples combined from the four sites for subsequent analyses. Phytoplankton were identified based on descriptions of Prescott (1978) and enumerated with an inverted microscope equipped with a calibrated micrometer (Kotak et al. 1995). Biovolume of each species was determined by estimating volumes from similar geometric shapes.

MC-LR analysis

In 1990 and 1991, phytoplankton for MC-LR analysis were concentrated from the lake surface (0–0.5 m) with a 64-μm-mesh plankton net. A minimum of five horizontal hauls were made per site. From 1992 to 1994, phytoplankton were collected by vertical hauls of the net over the same depth range for which water was collected for chemical analyses (0–2 or 0–1 m). Samples were dewatered (after combining the samples from the four sites) by passing them through a Plexiglas tube (15-cm height, 12-cm diameter) fitted with 64-μm-mesh Nitex netting on one end. The sample was then frozen immediately upon transport to the laboratory and later freeze-dried. Cells (approximately 50 mg) were extracted twice in 5% acetic acid and microcystins concentrated and purified on disposable C18 cartridges (Supelco Inc., Bellefonte, Pa.).

Samples were then analysed by reverse-phase high-performance liquid chromatography (HPLC) as follows. From 1990 to 1992, MC-LR was quantified on a Waters 6000A HPLC with an HP 1040A diode array detector and HP85 data processor (Kenefick et al. 1992); samples were run under isocratic conditions at a flow rate of 1 mL·min^{-1}. The mobile phase consisted of 10 mM ammonium acetate and acetonitrile (74:26%). In 1993 and 1994, MC-LR was analysed on a Waters LC Module I HPLC with a Waters model 746 data module. In both cases, MC-LR detection was

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based on ultraviolet absorbance at 238 nm. In 1993, samples were run under isocratic conditions at a flow rate of 1 mL·min^{-1}. The mobile phase consisted of 10 mM ammonium acetate and acetonitrile (76:24%). In 1994, samples were run under gradient conditions (20–30% acetonitrile in 10 mM ammonium acetate over 10 min and then 20% for the remainder of the run (30 min)) at a flow rate of 1 mL·min^{-1}. A comparison of the two HPLCs and the three separation methods (two isocratic, one gradient) indicated no significant difference between the quantity of MC-LR detected, although the gradient method gave much better separation of MC-LR from other interfering peaks (Kotak 1995). MC-LR concentration, initially calculated as micrograms per gram of phytoplankton biomass, was then reexpressed volumetrically as nanograms per litre (Kotak et al. 1995) and represented toxin concentrations within the cyanobacterial cells per litre of lake water (0–2 or 0–1 m depth), not as dissolved (aqueous) toxin. MC-LR as nanograms per litre was determined using the biomass of phytoplankton in the water (dry weight per litre) based on the published relationship between Chl *a* and phytoplankton dry weight (APHA et al. 1992). MC-LR concentration was also expressed as micrograms per gram of *M. aeruginosa* biomass by combining the MC-LR data (as nanograms per litre) and *M. aeruginosa* abundance (cells per litre) and scaling the quantity of toxin to a per gram of *M. aeruginosa* basis. This calculation assumed that all MC-LR was produced by *M. aeruginosa* (see Kotak et al. 1993, 1995; Discussion section in this paper). The detection limit for MC-LR by both HPLCs and the three methods of analysis was approximately 10–20 ng per 20-μL injection.

In 1990 and 1991, the sampling depth for MC-LR and phytoplankton identification and enumeration was different from the depth of collection of water chemistry data in all years and of phytoplankton data collected from 1992 to 1994 (0–2 or 0–1 m). Since all samples were collected in the middle of the lakes, where floating scums of cyanobacteria rarely occur, we assumed that phytoplankton species composition and MC-LR concentration would be similar between surface samples (0–0.5 m) and integrated (0–2 and 0–1 m) samples. This assumption was validated in 1992 (data not shown).

Statistics

To examine the correlation between MC-LR concentration, biomass of *M. aeruginosa*, limnological variables, and cyanobacterial species composition, all data from individual sampling dates were log transformed. This data set (referred to as the combined data) represented all 13 lake basins sampled in 1991 and up to six of the lakes sampled for the entire 5-year period. Similarly, the 1991 data set (representing individual sampling dates combined from all 13 lake basins in 1 year) was analysed by correlation analysis separately. To evaluate the correlation between MC-LR and variables that have a single value (i.e., mean and maximum lake depth, water residence time, and lake surface area to watershed area ratio), summer mean MC-LR was used for each lake. A univariate linear regression model was constructed based on log-transformed or untransformed combined data to predict MC-LR concentration in phytoplankton (nanograms per litre). SPSS for Windows statistical software (version 6.0) was used for all analyses.

Results

Study lakes

In 1991, all 13 lake basins were studied, and therefore, descriptions of the general limnology and seasonal patterns of MC-LR concentration in phytoplankton are made only for this year. All lakes were eutrophic to hypereutrophic based on mean summer TP and Chl *a* concentrations (Table 1). Lac La Nonne and Driedmeat and Little Beaver lakes had the

Table 1. Water quality variables and lake and watershed characteristics for the 12 study lakes.

Lake	$NO_3^- + NO_2^-$ (μg·L^{-1})	TP (μg·L^{-1})	Chl *a* (μg·L^{-1})	SO_4^{2-} (μequiv.·L^{-1})	Na^+ (μequiv.·L^{-1})	HCO_3^- (μequiv.·L^{-1})	Conductivity (μS·cm^{-1})	Z_{max} (m)	Z (m)	A_d (km^2)	A_o (km^2)	A_d:A_o	WRT (years)
Baptiste North	16	57	24	229	1175	3065	332	15.5	5.6	109	5.07	22	6.0
Baptiste South	12	35	12	250	1131	2983	329	27.5	11.9		4.74		
Coal	54	103	33	937	2349	3524	498	5.5	3.5	1250	10.9	115	0.9
Driedmeat	38	201	13	791	1697	3507	422	3.7	2.2	7220	16.5	438	0.3
Figure Eight	29	95	32	312	131	1950	249	6.0	3.0	4	0.4	10	4.5
Halfmoon South	13	71	26	208	957	1639	268	8.5	4.7	2.4	0.4	6	60.0
Hastings	19	87	41	5267	4133	4556	836	7.3	2.4	269	8.7	31	>100
Isle	16	124	62	146	783	2459	296	7.5	4.1	246	23	11	9.5
Lac La Nonne	17	213	79	229	827	2311	314	19.8	7.8	277	11.8	24	18.5
Little Beaver	34	186	49	2561	5307	4458	814	2.5	2.0		0.75		
Nakamun	12	102	71	125	957	2262	275	8.0	4.5	45	3.5	13	21.0
Steele	20	116	27	21	305	2737	265	6.1	3.2	255	6.6	39	2.0
Wizard	47	63	30	104	1131	2786	310	11.0	6.2	29	2.5	12	13.5

Note: Water quality variables represent means for May–September 1991 and are based on analysis of water samples collected from the surface to a depth of 2 m (or a depth of 1 m in Little Beaver Lake). Lake and watershed characteristics (maximum (Z_{max}) and mean lake depth (Z), watershed area (A_d), lake surface area (A_o), A_d:A_o, and water residence time (WRT)) were obtained from Mitchell and Prepas (1990). A_d:A_o and WRT for Baptiste Lake are from both basins combined and are unknown for Little Beaver Lake.

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highest mean summer TP concentrations, while Baptiste Lake had the lowest (Table 1). The highest mean summer Chl *a* concentration (79 $\mu g \cdot L^{-1}$) in the upper 2 m of the water column was recorded in Lac La Nonne, which also occasionally had surface (top 10 cm of the lake) Chl *a* concentrations exceeding 150 $\mu g \cdot L^{-1}$. Mean summer Chl *a* concentration in Nakamun and Isle lakes was also high (Table 1). The dominant ions in these hardwater lakes were Na^+, SO_4^{2-}, and HCO_3^- (Table 1), and mean summer conductivity ranged from 249 to 846 $\mu S \cdot cm^{-1}$. Estimated water residence was 0.3 to >100 years (Table 1). The lakes ranged from shallow to moderately deep (maximum depth from 2.5 to 27.5 m). Deeper lakes, such as Baptiste Lake, Lac La Nonne, and Wizard Lake, were thermally stratified by early summer, whereas shallower lakes (Little Beaver, Driedmeat, Coal, Figure Eight, and Steele) were either weakly stratified or mixed throughout the sampling period. The lakes therefore represented a cross section of eutrophic, hardwater, and primarily freshwater lakes on the boreal plains.

Occurrence and concentration of MC-LR

A total of 90 phytoplankton samples were collected from the 13 lake basins in 1991 and analysed by HPLC for MC-LR. Phytoplankton biomass was not sufficient to allow MC-LR analysis on several occasions in many of the lakes. Seventy percent of the samples collected (i.e., 63 out of 90) contained detectable concentrations of MC-LR (Fig. 1). More than one half of the lakes surveyed (Coal, Driedmeat, Halfmoon South, Hastings, Little Beaver, Nakamun, and Steele) contained detectable concentrations of MC-LR in more than 50% of the phytoplankton samples collected over the summer. In the remaining lakes, MC-LR was detected <25% of the time (Figure Eight and Isle lakes and Lac La Nonne) or only once (Baptiste North and South and Wizard) in the phytoplankton. All 13 lake basins had detectable concentrations of MC-LR at least once during 1991. In those lakes in which MC-LR was present in a high proportion of the samples collected over the summer, MC-LR concentration was generally highest during the latter part of the sampling period (Figs. 1*b* and 1*c*). The extreme case was Little Beaver Lake where MC-LR was highest from late August to early October (Fig. 1*c*). The highest MC-LR concentration detected in 1991 (6174 $ng \cdot L^{-1}$) was from phytoplankton collected from Little Beaver Lake on 9 October, when water temperature was only 8°C.

Lakes fell into three distinct categories based on MC-LR concentration averaged over the 1991 sampling period: low (<50 $ng \cdot L^{-1}$: Baptiste North and South, Wizard, and Figure Eight), intermediate (150 to 1000 $ng \cdot L^{-1}$: Coal, Isle, Driedmeat, Lac La Nonne, Steele, Hastings, and Halfmoon South), and high (>1500 $ng \cdot L^{-1}$: Nakamun and Little Beaver).

Relationship to environmental variables

MC-LR concentration in the phytoplankton (expressed as nanograms per litre) in the combined data set was correlated with TP, Chl *a*, Secchi depth, Na^+, color, pH, turbidity, and conductivity (Table 2). Many of the variables, such as Chl *a*, pH, and Secchi depth, however, are also autocorrelated. Of all the water chemistry variables, the strongest correlation was with TP (Fig. 2*a*). The strongest correlation between MC-LR and a cyanobacterial species was with *M. aeruginosa* ($r = 0.52$) (Table 2), one of the main producers of the toxin. There was no relationship between MC-LR concentration in the phytoplankton and *Anabaena flos-aquae*, the only other species that was present in the lakes that is capable of producing MC-LR (Table 2). MC-LR was also correlated to the biomass of *Phormidium* sp., probably a result of its correlation with *M. aeruginosa* ($r = 0.42$). *Phormidium* sp. is not known to produce MC-LR. Results of the correlation analyses based on 1991 data alone were consistent with the analyses based on the combined data (Table 2). The directions of the relationships were identical between the two data sets, although correlation coefficients between the combined and 1991 data sets were different (Table 2). In either data set, there was no relationship between MC-LR in the phytoplankton and SO_4^{2-}, HCO_3^-, and water temperature (Table 2) or any of the other ions (K^+, Ca^{2+}, Mg^+, and Cl^-; not shown). The relationships between MC-LR and inorganic nitrogen ($NO_3^- + NO_2^-$ and NH_4^+) in both data sets were not linear (Figs. 3*a* and 3*b*) and no transformation adequately linearized the data.

The strong relationship between *M. aeruginosa* biomass and MC-LR concentration in the phytoplankton community was expected, and we thus explored the hypotheses that MC-LR in phytoplankton might be influenced by factors that affect the biomass of *M. aeruginosa* produced or toxin production within *M. aeruginosa* cells or both.

Because MC-LR in these lakes was produced by *M. aerugionsa*, we also expressed MC-LR concentrations as micrograms per gram of *M. aeruginosa* biomass. Thus, we determined if the biomass of *M. aeruginosa* was correlated with the same limnological variables as those that affect MC-LR concentration in the species. Both *M. aeruginosa* biomass and cellular MC-LR (expressed as micrograms per gram of *M. aeruginosa*) were correlated with TP ($r = 0.45$ and 0.46, respectively) (Figs. 2*b* and 2*c*), Chl *a* ($r = 0.49$ and 0.33), Secchi depth ($r = -0.36$ and 0.32), and pH in the combined data set ($r = 0.34$ and 0.25) (Table 3). There were also nonlinear relationships between either *M. aeruginosa* biomass (Figs. 3*c* and 3*d*) or cellular microcystin concentration (Figs. 3*e* and 3*f*) and forms of inorganic nitrogen ($NO_2^- + NO_3^-$ and NH_4^+). However, whereas *M. aeruginosa* biomass was correlated with turbidity ($P = 0.008$), MC-LR concentration per unit biomass was not ($P = 0.15$). In addition, MC-LR (as micrograms per gram of *M. aeruginosa*) was correlated with conductivity and color ($P < 0.05$), whereas *M. aeruginosa* biomass was not ($P > 0.11$) (Table 3). Therefore, *M. aeruginosa* biomass was not necessarily correlated with the same limnological variables as MC-LR concentration per unit biomass of *M. aeruginosa*.

A linear regression between MC-LR in phytoplankton (as nanograms per litre) and log-normalized ratio of TN to TP (TN:TP) was significant ($P < 0.02$, df = 1, 36) and explained 70% of the variation in MC-LR (i.e., $r^2 = 0.70$). The original relationship between MC-LR and TN:TP, however, was not linear (Fig. 4*a*).

Discussion

MC-LR concentration in phytoplankton in lakes can be highly dynamic in terms of within- and between-year, diel, and spatial variation (Kotak et al. 1993, 1995, 1996). Much

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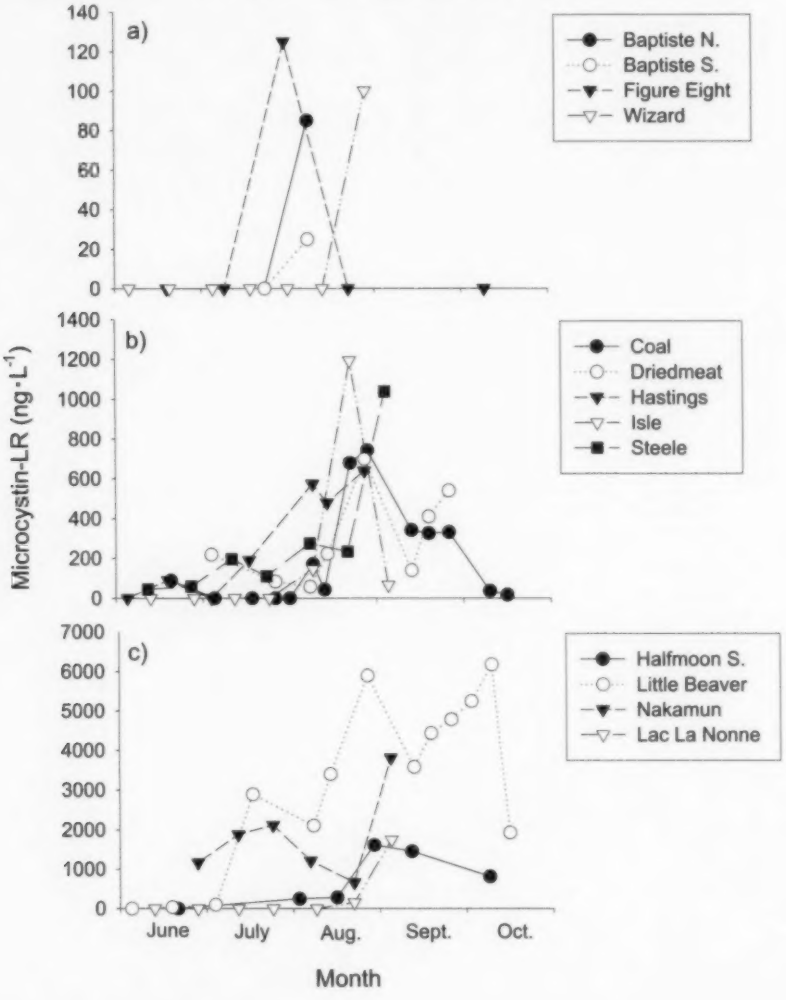
Fig. 1. MC-LR concentration in the phytoplankton from the 12 study lakes during 1991. MC-LR concentration represents cellular toxin, not dissolved in water. Phytoplankton samples for MC-LR analyses were collected with a plankton net from the lake surface (0–0.5 m). Lakes are divided into those with (*a*) low seasonal MC-LR concentration, (*b*) intermediate seasonal concentration, and (*c*) high seasonal concentration. Note the change in MC-LR scale between sets of lakes.



of the variability in seasonal changes in MC-LR in the phytoplankton from three hypereutrophic Alberta lakes (Coal, Driedmeat, and Little Beaver) was attributed to changes in the seasonal abundance and biomass of *M. aeruginosa*, the main producer of MC-LR (Kotak et al. 1995). In the present study, MC-LR concentration in the phytoplankton from a larger suite of study lakes was also strongly correlated with the biomass of *M. aeruginosa* ($r = 0.52$, based on the combined data). It is clear, however, that *M. aeruginosa* biomass only partly explains changes in MC-LR concentration in the phytoplankton. We believe that some of this residual variation may be explained by environmental factors that influence toxin production within *M. aeruginosa* cells as well as the occurrence of various strains of *M. aeruginosa* that produce no MC-LR (see discussion in Kotak et al. 1995). The presence in a critical mass of the producer may set the potential for MC-LR in mixed assemblages of phytoplankton, and the environmental factors may modify the MC-LR production.

Given the importance of *M. aeruginosa* abundance and (or) biomass in the total quantity of MC-LR in samples of mixed phytoplankton assemblages, it is important to understand the factors that regulate both the occurrence and the biomass of *M. aeruginosa* in lakes as well as toxin production within the *M. aeruginosa* cells. The factors that may regulate *M. aeruginosa* biomass/abundance in lakes need not be the same as those that regulate toxin production. To investigate this, we expressed MC-LR concentration on a per unit biomass basis of *M. aeruginosa* and compared the factors that were correlated with MC-LR with those correlated with *M. aeruginosa* biomass. Both the biomass of *M. aeruginosa* and cellular MC-LR (as micrograms per gram of

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Table 2. Correlations between MC-LR concentration ($ng \cdot L^{-1}$) and limnological variables and cyanobacterial species biomass based on log-transformed data from all data combined (1990–1994) and from 1991 data (June–October) only.

	Combined data			1991 data		
Variable	*r*	*P*	df	*r*	*P*	df
TP	0.54	***	254	0.38	***	86
Chl *a*	0.48	***	266	0.36	***	90
Secchi depth	–0.38	***	262	–0.43	***	88
Na+	0.34	***	89	0.29	*	51
Color	0.33	***	88	0.35	**	52
pH	0.32	***	228	0.28	**	86
Turbidity	0.30	***	119	0.34	***	83
Conductivity	0.21	***	180	0.29	**	86
SO_4^{2-}	0.16	ns	53	0.09	ns	47
$NO_3^- + NO_2^-$	–0.11	ns	156	–0.18	ns	86
HCO_3^-	0.07	ns	226	0.08	ns	83
NH_4+	0.05	ns	220	0.04	ns	84
Water temperature	0.00	ns	261	–0.16	ns	84
Microcystis aeruginosa	0.52	***	215	0.45	***	83
Phormidium sp.	0.52	***	214	0.45	***	82
Aphanizomenon flos-aquae	0.07	ns	214	–0.13	ns	82
Gomphosphaeria sp.	–0.05	ns	214	0.09	ns	82
Anabaena flos-aquae	–0.03	ns	215	0.10	ns	82

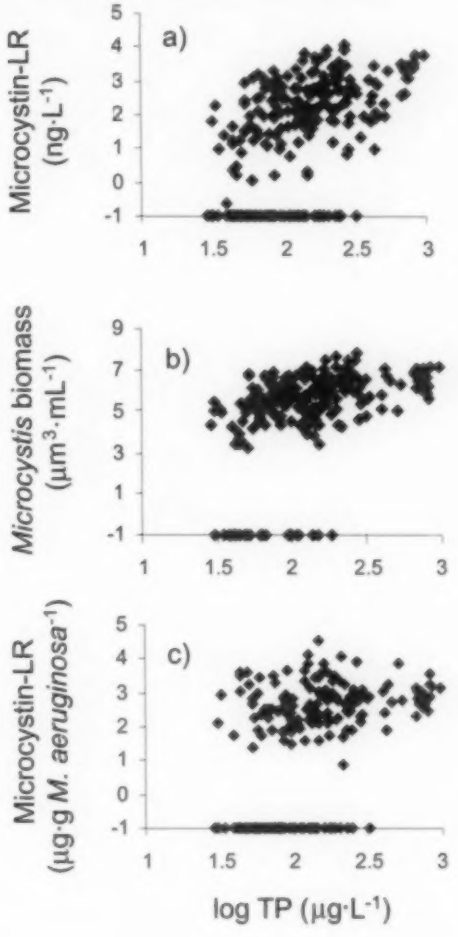
Note: $*P < 0.05$; $**P < 0.01$; $***P < 0.005$; ns, not significant ($P > 0.05$). df = degrees of freedom ($n - 2$).

M. aeruginosa) were strongly correlated with TP, Chl *a*, Secchi depth, and pH, suggesting that these variables may affect both *M. aeruginosa* biomass and toxin production per unit of *M. aeruginosa* biomass. However, turbidity, conductivity, Na^+, and color were correlated only with *M. aeruginosa* biomass or MC-LR, not both. Therefore, it is likely that certain limnological variables act directly on the growth (and hence the biomass produced) of *M. aeruginosa* and others on MC-LR production. Results from Van der Westhuizen and Eloff (1985) support this hypothesis, since conditions that were optimal for growth (e.g., water temperature >30°C) in laboratory strains of *M. aeruginosa* were not optimal for microcystin production.

The production of microcystins by cyanobacteria may also be greatly affected by the physiological condition of the cells. Van der Westhuizen and Eloff (1985) found that microcystin production was tightly coupled to the cell cycle in laboratory *M. aeruginosa* strains, with maximum toxin production occurring in late log phase. We found a correlation between MC-LR concentration in *M. aeruginosa* and *M. aeruginosa* biomass ($r = 0.46$). If we use biomass as a rough indicator of stage in the life cycle, then maximum MC-LR concentration in our study also occurred during periods of maximum growth (i.e., late exponential growth phase).

The mechanisms by which environmental factors affect toxin production by cyanobacteria are poorly understood. Although nutrients have long been suspected as a contributing factor to both cyanobacterial abundance and toxin production, surprisingly little information has been published on how, for example, changes in phosphorus and nitrogen may affect microcystin production.

Fig. 2. Relationship between log TP and (*a*) MC-LR concentration, (*b*) *M. aeruginosa* biomass, and (*c*) MC-LR concentration.



In our study, phosphorus was the nutrient most strongly correlated with MC-LR concentration in the phytoplankton. This is consistent with our previously report of Coal, Driedmeat, and Little Beaver lakes from 1990 to 1992 (Kotak et al. 1995). TP in our present study was a correlate of MC-LR concentration expressed on both a nanogram per litre and a microgram per gram of *M. aeruginosa* basis. In contrast, Watanabe and Oishi (1985) found little change in the toxicity of *M. aeruginosa* under culture conditions when phosphorus concentration in the media was reduced to 1/10 and 1/20 of the original culture concentration; however, the original concentration of phosphorus was extremely high (100 mg orthophosphate·L^{-1}) compared with natural freshwaters, and even at 1/20 of the original concentration, phosphorus was probably not limiting. In contrast with the above study, Sivonen (1990) found that orthophosphate concentration had a marked effect on [D-Asp^3]microcystin-RR production in laboratory cultrues of *Oscillatoria agardhii* at low phosphorus concentrations (100–400 µg·L^{-1}) but not at higher phosphorus concentrations.

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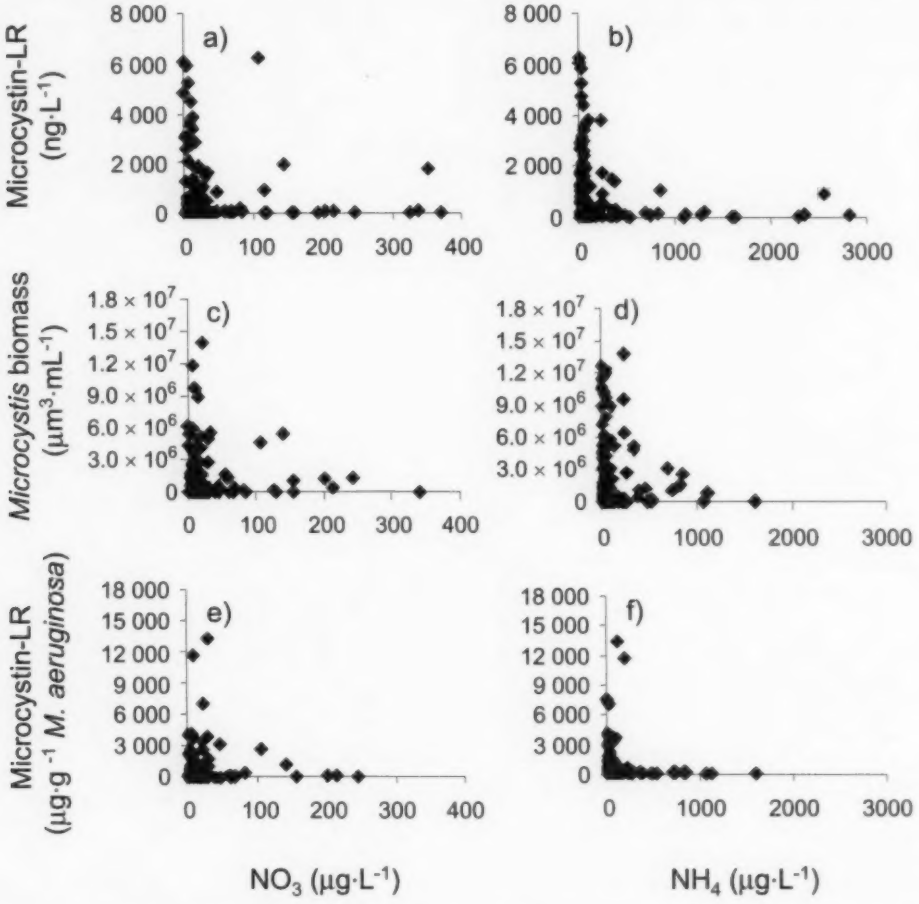
Fig. 3. Relationship between NO_3^- + NO_2^- and (*a*) MC-LR concentration, (*c*) *M. aeruginosa* biomass, and (*e*) MC-LR concentration and between NH_4^+ and (*b*) MC-LR concentration, (*d*) *M. aeruginosa* biomass, and (*f*) MC-LR concentration. Graphs are based on the combined (1990–1994) data.



There was a strong negative relationship between MC-LR concentration and NO_3^- + NO_2^- and NH_4^+. We found a similar pattern when examining data from Coal, Driedmeat, and Little Beaver lakes from 1990 to 1992 (Kotak et al. 1995). Despite the lack of linear association, it is evident that maximum MC-LR concentration occurred when sources of inorganic nitrogen were at their seasonal lowest in the lakes (<10 $\mu g \cdot L^{-1}$ for NO_3^- + NO_2^- and <30 $\mu g \cdot L^{-1}$ for NH_4^+). Rapala et al. (1993) examined the effect of nitrogen concentration and source (N_2, NO_3^-, NH_4^+, and urea) on the production of the cyanobacterial neurotoxin anatoxin-a in several laboratory strains of *Aphanizomenon* and *Anabaena*. They found that toxin production was highest when cells were grown in nitrogen-free media and was lowest when grown in nitrogen-rich media. However, both genera are capable of N_2 fixation in oxic environments, whereas *Microcystis* is not. In contrast, Sivonen (1990) found that production of [D-Asp^3]microcystin-RR in *O. agardhii* increased with increasing NO_3^- concentration. The differences in the response of toxin production to nitrogen or phosphorus manipulation is not surprising because the response might be expected to be species dependent. Additionally, the function of anatoxin-a and microcystins in the cyanobacteria may be very different, and therefore, the response to changes in nitrogen and phosphorus would be expected to differ.

The absolute concentration of TP and TN:TP have been suggested to affect cyanobacterial abundance (Smith 1983; Trimbee and Prepas 1987). We found that during the summer of 1993, MC-LR concentration in the phytoplankton was dramatically reduced when TN:TP was >5:1. In regression analysis, MC-LR concentration in phytoplankton was also best predicted from TN:TP. The impact of TN:TP on MC-LR concentration in the phytoplankton was manifested mainly by the affect of TN:TP on *M. aeruginosa* biomass but not on MC-LR concentration in *M. aeruginosa*.

The negative relationship between inorganic nitrogen and *M. aeruginosa* biomass was unexpected, as heterocystous species of cyanobacteria generally dominate phytoplankton communities in low inorganic nitrogen environments, due to their ability to fix atmospheric nitrogen. Blomqvist et al. (1994) indicated that non-nitrogen-fixing cyanobacterial spe-

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Table 3. Correlations between MC-LR concentration (μg·g *M. aeruginosa*$^{-1}$) and *M. aeruginosa* biomass (μm^3·mL^{-1}) and limnological variables based on log-transformed combined data.

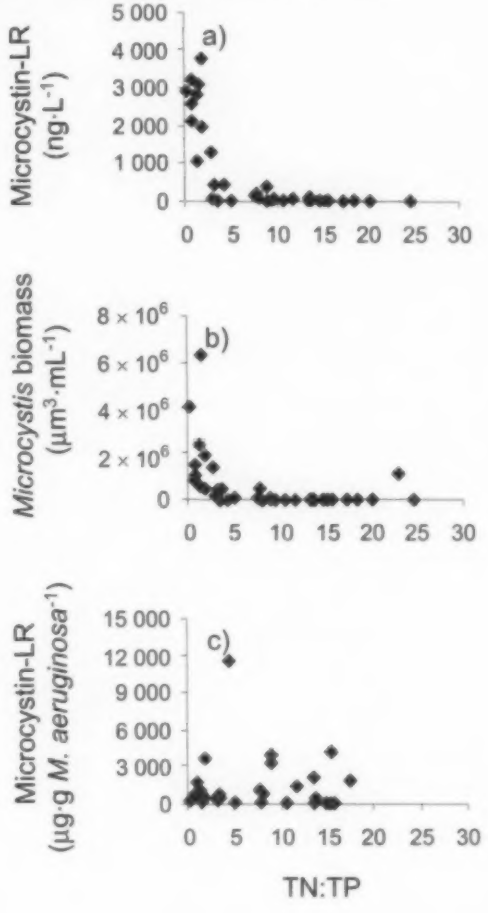
	MC-LR concentration			*M. aeruginosa* biomass		
Variable	*r*	*P*	df	*r*	*P*	df
Secchi depth	–0.32	***	207	–0.36	***	236
TP	0.46	***	206	0.45	***	236
Chl *a*	0.33	***	209	0.49	***	237
Color	0.28	*	53	0.21	ns	60
Turbidity	0.16	ns	81	0.27	**	94
Na+	0.26	ns	52	0.09	ns	60
Conductivity	0.27	***	138	0.02	ns	159
pH	0.25	***	183	0.34	***	211
$NO_3^- + NO_2^-$	–0.06	ns	114	0.04	ns	134
Water temperature	–0.12	ns	204	0.06	ns	234
SO_4^{2-}	0.12	ns	42	0.07	ns	47
HCO_3^-	0.08	ns	182	0.07	ns	210
NH_4+	0.02	ns	182	0.03	ns	207
M. aeruginosa	0.46	***	210			

Note: *$P < 0.05$; **$P < 0.01$; ***$P < 0.005$; ns, not significant ($P > 0.05$). df = degrees of freedom ($n - 2$).

cies such as *Microcystis* commonly dominate phytoplankton communities in lakes when inorganic nitrogen is low, due to their superior competitiveness for NH_4^+. Our data suggest that microcystins may function as an internal store of nitrogen for use during periods of low nitrogen in the environment (i.e., under periods of nitrogen stress). This ecological/physiological role for microcystins warrants investigation, by examining cellular N:P and microcystin production in a combination of laboratory experiments and field surveys.

Light intensity also has a marked effect on MC-LR production under culture conditions. Generally, microcystin production is highest at lower light intensities (Van der Westhuizen and Eloff 1985; Watanabe and Oishi 1985; Utkilen and Gjolme 1992). We found that MC-LR concentration in the phytoplankton and turbidity and color were correlated in the present study, suggesting that MC-LR production was higher under lower light conditions in lakes. However, the relationships were much weaker than those for phosphorus and nitrogen. The negative correlation between MC-LR in phytoplankton and Secchi depth in our study (r = –0.34 to –0.43) also supports the hypothesis that light may influence MC-LR. However, closer examination of the data suggests that turbidity affected MC-LR concentration in the phytoplankton mainly by its affect on *M. aeruginosa* biomass and not on the concentration of the toxin in the *M. aeruginosa* cells and that color may influence the concentration of MC-LR in the cells but not *M. aeruginosa* biomass. These contradictory results emphasize that correlations drawn between environmental variables and those associated with gas-vacuolate species such as *M. aeruginosa* should be interpreted with caution because these species are able to rapidly change their position in the water column (Reynolds 1987). The limnological conditions (light, nutrients, water temperature) that the colonies are exposed to will also change rapidly, particularly in deep stratified lakes (e.g., in four of the 13 lake basins in this study).

Fig. 4. Relationship between (*a*) MC-LR concentration, (*b*) *M. aeruginosa* biomass, and (*c*) MC-LR concentration and TN:TP. Data represent individual sampling dates during 1993 for Coal, Driedmeat, and Little Beaver lakes combined.



Mean summer MC-LR in 1991 was negatively correlated with mean and maximum lake depth (r = –0.50 and –0.56, respectively). This should not be surprising if *M. aeruginosa* abundance/biomass is an important factor controlling the concentration of MC-LR in the water column. Genera such as *Microcystis* tend to be more prominent in shallow lakes that can turn over rapidly due to wind-induced turbulence (Reynolds 1987). We found, however, no relationship between MC-LR concentration and water residence time (range 0.3 to >100 yearrs) or the ratio of watershed area to lake surface area (a surrogate for estimating potential external nutrient load). This was expected because internal phosphorus loading commonly makes up a high proportion of, if not almost the entire, TP load to the euphotic zone on an annual basis in central Alberta lakes (Shaw and Prepas 1990; D.O. Trew and B.G. Kotak, unpublished data). These relationships should be interpreted with caution, however, because use of seasonal means based on a limited number of samples greatly reduces the power of the statistical test.

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A study of more limited scope (Kotak et al. 1995) on Coal, Driedmeat, and Little Beaver lakes over a shorter period (1990–1992) yielded correlations between MC-LR concentration in phytoplankton and variables such as *M. aeruginosa* biomass, Chl *a*, TP, Secchi depth, and pH, a similar negative relationship between MC-LR and either $NO_3^- + NO_2^-$ or NH_4^+, and no relationship between MC-LR and water temperature based on monthly means that are consistent with the larger data set assembled in the present study. The lack of relationship between MC-LR and water temperature is intriguing because, although not explicitly stated in the literature, maximum cyanobacterial toxin concentration in phytoplankton is often assumed to occur during the summer when water temperatures are at their highest. We have recorded seasonal high toxin concentrations in Driedmeat and Little Beaver lakes in spring and autumn when water temperatures were below 10°C (Kotak et al. 1995). The occurrence of neurotoxic blooms of *A. flos-aquae* in ice-free American Lake, Washington, during the winter by Jacoby et al. (1994) supports the premise that toxin-producing blooms are not restricted to warm summer months.

Wicks and Thiel (1990) also attempted to correlate microcystin concentrations in phytoplankton with environmental variables. They found that microcystin concentration (total of six microcystin analogues) in phytoplankton of Hartbeespoort Dam, South Africa, over 2.5 years was positively correlated with biomass-normalized primary production, solar radiation, oxygen saturation of the water, water temperature, and pH and was negatively correlated with Chl *a* and orthophosphate concentrations. However, the applicability of their data to north-temperate lakes is questionable because (*i*) water temperature in Hartbeespoort Dam did not decrease below 15°C during the winter months (unlike some north-temperate lakes that may be ice-covered for >6 months) and (*ii*) nutrient concentrations in the reservoir are almost always well above the requirements for phytoplankton. In addition, sample sizes in their correlation analyses were small (n = 16–20, depending on the variable).

Our data suggest that an increase in phosphorus loading (whether internally or externally mediated) and a shift in N:P may increase the incidence of toxic blooms of cyanobacteria and the concentration of toxin produced, with respect to MC-LR and *M. aeruginosa*. This study corroborates the limited information available from laboratory culture experiments that indicate the importance of nitrogen and phosphorus in MC-LR production. It is also the first study to relate MC-LR concentrations in phytoplankton based on data collected from several lakes over a relatively long time frame (5 years) to environmental variables. This needs to be done in other regions of the world to test the generality of the relationships presented here.

In conclusion, there was a wide range in MC-LR concentration in the phytoplankton between the study lakes, and the concentration of MC-LR was probably regulated by both the biomass of *M. aeruginosa* in the water column and the concentration of MC-LR produced per unit mass of *M. aeruginosa*. Factors such as TP may have had an impact on both *M. aeruginosa* abundance and MC-LR produced per unit mass of the species, while factors such as TN:TP may have affected *M. aeruginosa* abundance only. Understanding the mechanisms of how limnological variables affect microcystin production at the cellular level is needed.

For example, a preliminary study in our laboratory utilizing *M. aeuginosa* PCC7820 indicated that decreasing N:P in growth media had a profound stimulatory effect on growth, confirming our field results (B.G. Kotak, G. van Tegham, and E.E. Prepas, unpublished data). Volumetric MC-LR concentration (nanograms per litre) in each flask also increased with decreasing N:P, due to the effects of *M. aeruginosa* biomass accumulation. In contrast, however, MC-LR concentration per cell decreased precipitously with decreasing N:P. This may suggest that MC-LR might function as a nitrogen source during growth and (or) under nitrogen limitation. MC-LR was present in high concentration in the cell when nitrogen was not limited (but growth rate and biomass were low, and thus, overall volumetric concentration of the toxin was low) but was depleted in the cell as growth became exponential (or when external nitrogen was depleted). To confirm the hypothesis that MC-LR may be utilized as an energy source, the fate of the carbon atoms in the MC-LR structure under exponential growth conditions needs to be examined. It is unlikely that the large decrease that we observed in the concentration of MC-LR per cell during the growth phase was due to loss or release of the toxin to the surrounding growth media. Previous studies have shown that MC-LR is an endotoxin and is released to the water phase only during massive cell lysis (which would not occur during growth phase) or after chemical treatment that compromises the cell wall. Further, our laboratory results also suggest that MC-LR production is not a response to nitrogen limitation stress because MC-LR production per cell was dramatically lower when N:P in the growth media was 0.9:1 compared with 9:1 and 18:1.

It appears that measured concentration of MC-LR in natural phytoplankton communities is a balance between biomass of the producer and cellular production of the toxin and that conditions that may be optimal for growth may not be optimal for toxin production. These hypotheses should ideally be evaluated in field studies such as the present one, mesocosm experiments (e.g., lake enclosures), and laboratory experiments with cells isolated from the same enclosures and lakes. Perhaps our best approach to understanding how microcystin production is affected by limnological variables is to answer other related questions such as why are microcystins produced and what is their function. This would go a long way toward our understanding of how microcystin production is regulated in lakes.

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Population dynamics of rainbow smelt (*Osmerus mordax*) in Lakes Ontario and Erie: a modeling analysis of cannibalism effects

Brian F. Lantry and Donald J. Stewart

Abstract: We used a stochastic stage-based matrix model (annual time step) and a bioenergetics model (daily time step) to simulate population dynamics, production, consumption, and conversion efficiency for rainbow smelt (*Osmerus mordax*) populations in Lakes Ontario and Erie. Cannibalism on young-of the-year (YOY) smelt by yearlings was the only scenario that reproduced alternate-year recruitment cycles observed in Lakes Ontario and Erie. Assuming constant survivorship and 5% variation in cannibalism, less than one YOY consumed per yearling smelt per year in both lakes could produce fluctuations greater than those observed. We found that at estimated daily mortality rates and during the pelagic phase of larvae only, 2% of the yearling smelt in Lake Erie and 5.1% in Lake Ontario need to consume one YOY per day to induce the observed abundance fluctuations. Bioenergetics simulations of alternating recruitment produced fluctuations in simulated values for annual gross production of approximately 6–7 and 31–59% for the Lake Ontario and Lake Erie smelt populations, respectively.

Résumé : Nous avons employé un modèle matriciel stochastique à étages (intervalle de 1 an) et un modèle bioénergétique (intervalle d'un jour) pour simuler la dynamique de la population, la production, la consommation et l'efficacité de conversion des populations d'éperlan arc-en-ciel (*Osmerus mordax*) des lacs Ontario et Érié. Le cannibalisme des jeunes de 1 an an sur les jeunes de l'année était le seul scénario qui reproduisait les cycles de recrutement de 1 an sur deux observés dans les lacs Ontario et Érié. Si l'on pose que la survie est constante et que la variation du cannibalisme est de 5%, la consommation de moins d'un jeune de l'année par chaque éperlan d'un an au cours de l'année dans les deux lacs pouvait produire des fluctuations plus grandes que celles qui ont été observées. Nous avons découvert que, aux taux estimés de mortalité quotidienne, et seulement pendant la phase pélagique des larves, il suffisait que 2% des éperlans de 1 an du lac Érié et 5,1% de ceux du lac Ontario consomment un jeune de l'année par jour pour que cela induise les fluctuations observées de l'abondance. Les simulations par la bioénergétique des alternances de recrutement ont produit des fluctuations (valeurs simulées) de la production brute annuelle d'environ 6–7 et 31–59% respectivement pour les populations d'éperlans du lac Ontario et du lac Érié.

[Traduit par la Rédaction]

Introduction

Rainbow smelt (*Osmerus mordax*) are important prey fish to pelagic piscivores in all five of the Great Lakes (Brandt 1986; Henderson and Nepszy 1989; Lantry and Stewart 1993). Great Lakes smelt stocks have been subject to massive dieoffs, and recruitment has been highly variable (e.g., Nsembukya-Katuramu et al. 1981; Schaefer et al. 1981). Coincident with stocking of salmonids and recovery of walleye (*Stizostedion vitreum*) stocks, these smelt populations experienced reductions in size-at-age and removals of older age-classes (Christie et al. 1987; O'Gorman et al. 1987; Henderson and Nepszy 1989). Elimination of more fecund older age-classes threatens the resiliency of these populations to buffer recruitment variability and catastrophic losses resulting from year-class failures or mass dieoffs. In recent years, declines in smelt numbers and growth rates have occurred in Lakes Ontario and Erie (Christie et al. 1987; Henderson and Nepszy 1989), and similar trends are apparent from the upper lakes. In light of the competing demands on Great Lakes prey fishes coupled with declines in pelagic productivity related to decreased phosphorus loading (Scavia et al. 1986; Hartig et al. 1991; Makarewicz and Bertram 1991) and the proliferation of zebra mussels (*Dreissena polymorpha*) (Leach 1993; Nicholls and Hopkins 1993), continued evaluation of the mechanisms influencing recruitment variability is warranted.

In many fish communities, cannibalism exerts a strong influence on year-class strength (Forney 1976; Evans and Loftus 1987; Hobson and Welch 1995), but few instances of cyclic population behavior have been directly linked to this type of predation. He and LaBar (1994) in their simulation of the Lake Champlain smelt population found that cannibalism was most important in determining long-term mean population size. They found that cannibalism by age-1 to age-5 smelt aimed at yearlings (age 1) and young-of-the-year (YOY) produced cyclic abundance in adults with a period of

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B.F. Lantry[1] and D.J. Stewart. College of Environmental Science and Forestry, State University of New York, 1 Forestry Drive, Syracuse, NY 13210, U.S.A.

[1]Author to whom all correspondence should be sent at the following address: New York State Department of Environmental Conservation, Cape Vincent Fisheries Station, P.O. Box 292, Cape Vincent, NY 13618, U.S.A.
e-mail: blantry@imcnet.net

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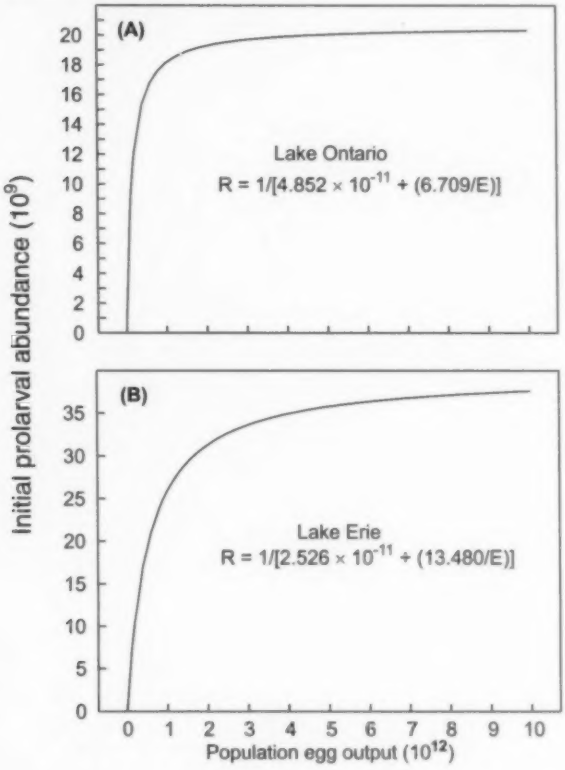
Fig. 1. Beverton and Holt (1957) density-dependent recruitment functions used to limit YOY rainbow smelt output for the simulated Lakes Ontario and Erie smelt populations. R is the number of prolarval smelt produced. α is equal to the inverse of maximum recruitment (4.852×10^{-11} for Lake Ontario and 2.526×10^{-11} for Lake Erie) and was derived from population fecundities in Lantry and Stewart (1993). β is the inverse of the recruitment rate per spawner (6.709 for Lake Ontario and 13.480 for Lake Erie) and was set to the inverse of the number of prolarvae divided by the number of adult spawners for each lake in Lantry and Stewart (1993).



4 years. When they confined cannibalism to only age-2 adults, a 2-year cycle in the abundance of YOYs and adults was evident. Lake Ontario and Lake Erie rainbow smelt populations have exhibited pronounced cycles of alternating year-class strength that also seem to be linked to cannibalism (O'Gorman et al. 1987; Henderson and Nepszy 1989). Predation by yearling smelt upon YOY conspecifics is a conceivable mechanism in the development of this population behavior (O'Gorman et al. 1987; Henderson and Nepszy 1989). Empirical evidence of this type of cannibalism is, however, lacking (Evans and Waring 1987; Henderson and Nepszy 1989). Rapid digestion of larval fish in the stomachs of predators and an exaggerated expectation of the amount of cannibalism necessary to induce observed abundance fluctuations may contribute to this paucity of evidence.

Our objectives in this study were to test the hypotheses that (*i*) cannibalism by yearling smelt on YOYs was the cause of the alternating years of recruitment strength observed for populations in Lakes Ontario and Erie and (*ii*) such 2-year cycles alter the production, consumption, and conversion efficiency of each population. To simulate recruitment patterns, we constructed a stage-based matrix model for smelt populations in Lakes Ontario and Erie from abundances and mortalities in Lantry and Stewart (1993). We used this model to evaluate the effects of cannibalism and variable survivorship within these smelt populations. Deterministic and stochastic simulations were implemented with different levels of cannibalism and natural mortality to evaluate alternative mechanisms that could approximate observed population cycles. We then used a rainbow smelt bioenergetics model (Lantry and Stewart 1993) to simulate the effect of alternating recruitment on production, consumption, and conversion efficiency.

Methods

Populations dynamics

We constructed an age-structured matrix model to simulate the dynamics of alternating year-class strength in populations of rainbow smelt from Lakes Ontario and Erie. The model was run with an annual time step for yearling and adult smelt cohorts. The first cohort of smelt was divided into egg and YOY stages that together represented 1 year of life. Matrices were set up with initial abundances and mortalities from Lantry and Stewart (1993, their table 1). We assumed a 1:1 sex ratio for both populations and obtained age-specific maturity rates from Chen (1972, their table 15). Fecundities (f_i) were calculated for females age 2 and older using the average total length (TL) at annulus for each age-class (Lantry and Stewart 1993) and the length – fecundity relationship from Dunstall (1980):

$$\log_{10}(f_i) = 2.97 \log_{10}(\mathrm{TL}) - 2.43.$$

Within model simulations, density-dependent control of recruitment was necessary to keep smelt numbers from rising exponentially towards infinity. There was, however, little evidence to suggest a particular type of density-dependent recruitment relationship for Great Lakes smelt. Jones et al. (1993) fitted a Shepherd (1982) function to yearling smelt abundance in Lake Ontario for 1978–1990. They indicated, however, that this function provided a poor fit to those data. The apparent decline in recruitment after 2000 t (Jones et al. 1993, their fig. 3) is due to the 1986 data point, which was by far the greatest number of yearlings produced, and then a series of data points from low-recruitment years. If cannibalism on YOYs by yearling smelt is the cause of alternating recruitment in Lake Ontario, then years of high and low recruitment that follow one another will be autocorrelated. This is because the number of yearling smelt produced in any year will be directly related to the number of YOYs consumed by yearlings in the previous year. To correct for this suspected autocorrelation, we plotted the Jones et al. (1993) data separately for alternate years of strong and weak smelt recruitment for Lake Ontario. No stock–recruitment relationship was evident from either plot. Because recruitment to the yearling stage was not correlated with parental stock size in Lake Erie (Henderson and Nepszy 1989) and the existence of a stock–recruitment relationship for Lake Ontario was questionable, we chose to be conservative and use a density-dependent function without a declining portion. To simulate recruitment of smelt prolarvae at the beginning of the YOY stage from eggs, we used the Beverton and Holt (1957) function with an asymptotic relationship between adult stock and recruitment (Fig. 1):

$$(1) \qquad R = 1/[\alpha + (\beta/E)]$$

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Table 1. Starting life history parameters for matrix model simulations of rainbow smelt populations in Lakes Ontario and Erie.

Stage	Stable age distribution	Survivorship	Fecundity (eggs/female)
Lake Ontario			
Egg	2.617×10^{12}	0.010	
YOY	2.617×10^{10}	0.602	
Age 1	8.062×10^{9}	0.465	
Age 2	4.853×10^{9}	0.121	3 010.7
Age 3	2.257×10^{9}	0.140	6 519.7
Age 4	2.731×10^{8}	0.140	8 386.3
Age 5	3.823×10^{7}	0.140	14 088.8
Six	5.352×10^{6}	0.000	
Lake Erie			
Egg	1.236×10^{12}	0.010	
YOY	1.236×10^{10}	0.308	
Age 1	3.808×10^{9}	0.256	
Age 2	9.749×10^{8}	0.251	15 375.3
Age 3	2.447×10^{8}	0.134	18 026.8
Age 4	3.279×10^{7}	0.010	27 323.6
Age 5	3.279×10^{5}	0.000	

Note: Age-6 smelt in Lake Ontario and age-5 smelt in Lake Erie simulations do not produce eggs.

where R is the number of YOYs (beginning at the prolarval stage), α and β are constants, and E is the number of eggs produced. In our model, simulated egg production (E) was determined according to

$$E = (0.5 \sum_{i=2}^{m} N_{i,t-1} A_i f_i)$$

where i is age-class, m is the last spawning age-class (age 5 in Lake Ontario and age 4 in Lake Erie), t is time, $N_{i,t}$ is the number of smelt of age i at time t, A_i is the proportion of the population that is mature at each age (Chen 1972), f_i is the size-specific fecundity rate, and 0.5 is the proportion of females in the population.

We determined β and R_{max} values from data in Lantry and Stewart (1993, their table 3). Because our stage-based model begins at the prolarval stage, we calculated β as the inverse of the number of prolarvae per adult. This resulted in values for β that yield approximately density-independent mortality (99% of eggs produced; Rupp 1965) at egg production values below R_{max} and density-independent and -dependent mortality values above R_{max}. Values for β, 6.709 for Lake Ontario and 13.48 for Lake Erie, were calculated from values from table 3 of Lantry and Stewart (1993) by dividing the number of prolarvae by the sum of all smelt (both sexes) age 2 and older up to the last spawning age-class (m). Initial prolarval abundance was calculated from table 3 of Lantry and Stewart (1993) as the population fecundity multiplied by an egg survivorship value of 0.01 from Rupp (1965). A detailed description of YOY, yearling, and adult abundance derivation is given in the appendix of Lantry and Stewart (1993). The last age-class in both lakes, age 6 in Lake Ontario and age 5 in Lake Erie, does not survive to spawning in model simulations. Since little is known about the maximum possible production of smelt recruits (R_{max}) for either Lake Ontario or Lake Erie, we arbitrarily set these values to the initial prolarval abundances. This yielded α values of 4.955×10^{-11} for Lake Ontario and 2.971×10^{-11} for Lake Erie. This strategy tended to constrain both smelt populations to levels at or below abundances given in Lantry and Stewart (1993). By assuming a density-dependent production of prolarvae, it was then possible to simulate varying levels of predation on YOY smelt and to evaluate effects on recruitment of both YOY to yearling and yearling to adult fish. While there are no data available to gauge the accuracy our estimates for R_{max} or prolarval abundances, our model is designed to evaluate population behavior in response to cannibalism and is more dependant on age-class structure than absolute abundance values.

Because it was not possible to calculate stable age distributions from our projection matrix, we estimated them with deterministic simulations run for 50 yearly time steps without cannibalism for each lake. We then adjusted α values slightly to 4.852442×10^{-11} for Lake Ontario and 2.52587×10^{-11} for Lake Erie to give YOY abundances equivalent to prolarval values in Lantry and Stewart (1993). Next, we repeated the deterministic simulations to attain final estimates of stable age distributions for all cohorts. We used the resulting age-class abundances from these estimated stable age distributions (Table 1) as initial values for all further simulations.

Simulated abundance of yearling smelt was dependent on cannibalism and was simulated in three ways.

(*i*) Cannibalism was simulated as an independent source of mortality reducing YOY numbers after natural mortality:

$$(2) \qquad N_{1,t} = [N_{0,t-1}(P_i S_p)] - \sum_{i=1}^{d} (N_{i,t-1} S_c X_i)$$

where d is the last age-class present (age 6 in Lake Ontario and age 5 in Lake Erie), $N_{i,t}$ is the number of smelt of age i at time t, P_i is the proportion surviving from age i to age $i + 1$, S_p is the stochasticity coefficient for survivorship, S_c is the stochasticity coefficient for cannibalism, and X_i is the number of YOY smelt eaten per smelt of age i, set to zero if an age-class has no cannibalism.

(*ii*) Cannibalism was simulated as an independent source of mortality reducing YOY numbers previous to natural mortality:

$$(3) \qquad N_{1,t} = (P_i S_p)[N_{0,t-1} - \sum_{i=1}^{d} (N_{i,t-1} S_c X_i)].$$

(*iii*) Cannibalism was simulated as a simultaneous component of natural mortality

$$(4) \qquad N_{1,t} = N_{0,t-1} e^{-(Z_c + Z_n)}$$

where

$$Z_n = -\log_e[1 - (P_i S_p)]$$

was the instantaneous natural mortality coefficient and

$$Z_c = -\log_e\{1 - [(\sum_{i=1}^{d} (N_{i,t-1} S_c X_i))/N_{0,t-1}]\}$$

was the instantaneous cannibalism mortality coefficient.

Simulated abundances for ages 2–5 were determined as

$$(5) \qquad N_{i,t} = N_{i-1,t-1} (P_i S_p)]$$

Stochasticity was implemented with the survivorship and cannibalism parameters to produce coefficients of variation of 5% for the cannibalism coefficient and 5, 10, and 20% for the survivorship coefficient. Values were determined by randomly selecting numbers within a normal distribution with a mean of 1 and a variance of 0.000 650 8 for the cannibalism coefficient and variances of 0.000 650 8, 0.002 603, and 0.029 9 for the survivorship coefficients. Cannibalism stochasticity was kept to a minimum because we did not want to hamper our ability to detect cannibalism-influenced cycles, because we were altering cannibalism levels between model simulations to evaluate the inducement of recruitment cycles, and because stochasticity was already implemented in the survivorship of all age-classes.

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Table 2. Lakes Ontario and Erie rainbow smelt population age-class structure used in bioenergetics simulations of alternating year-class strength.

YOY	Yearling	Age 2	Age 3	Age 4	Age 5
Lake Ontario					
1.061×10^{10}					
1.061×10^{10}	1.089×10^{9}				
	5.444×10^{9}	**6.557×10^{8}**			
		3.279×10^{9}	3.050×10^{8}		
			1.525×10^{9}	**3.682×10^{7}**	
				1.841×10^{8}	5.155×10^{6}
					2.578×10^{7}
Lake Erie					
3.036×10^{10}					
3.036×10^{10}	9.489×10^{8}				
	1.423×10^{10}	**2.431×10^{8}**			
		3.646×10^{9}	6.094×10^{8}		
			9.141×10^{8}	**8.166×10^{7}**	
				1.225×10^{8}	

Note: Diagonals represent the abundance of one year-class of smelt, at each age, throughout its life span. The upper diagonal has low first year survivorship (Lake Ontario = 10.3%, Lake Erie = 3.1%); the lower diagonal has high first year survivorship (Lake Ontario = 51.3%, Lake Erie = 46.9%). Boldfaced values are the age-specific abundances used in the bioenergetics simulation of the smelt population within a calendar year when first year mortality was high. Values not boldfaced are the age-specific abundances used in the bioenergetics simulation of the smelt population within a calendar year when first year mortality was low.

We estimated age-specific adult cannibalism levels by calculating the percent frequency of occurrence of fish in their diet (2% for age-2 smelt and 6% for age-3 and older smelt) from our earlier Lakes Michigan and Ontario empirical data (Lantry 1991) and assuming that all fish eaten were smelt. In that study, most fish eaten by adults were YOY smelt that had completed transformation from the larval to the juvenile stage. Studies of the distribution of larval smelt (<25 mm) in Lake Michigan indicate that after hatching and before transformation, larvae were widely dispersed in water from 10 to 16°C often in depths above the range normally inhabited by adults (Brandt et al. 1980; Tin and Jude 1983). In August, after transformation from the larval to the juvenile stage, YOY smelt concentrate along the bottom inshore at temperatures of 8–15°C (Tin and Jude 1983; Urban and Brandt 1993), increasing their vulnerability to cannibalism by adults. We calculated daily cannibalism by multiplying the percent frequency of occurrence of fish in the diet of adult smelt by their age-specific abundances corrected for daily mortality. We summed daily values from August 1 to May 31 the following year for each age-class to obtain the total number of YOYs cannibalized by each adult smelt age-class. We divided the total annual number of YOYs cannibalized for each age-class by their respective initial abundances to get individual adult smelt cannibalism rates for our stage-based model that ran on an annual time step. Adult smelt cannibalism rates were 3.85 YOYs per age-2 individual per year and 6.2 YOYs per individual aged 3–6 per year for Lake Ontario and 2.8 YOYs per age 2, 8.3 YOYs per age 3, 6.1 YOYs per age 4, and 0.44 YOY per age 5 per year for the Lake Erie population.

We simulated various combinations of smelt age-classes (yearling and older) preying on YOY smelt to determine if cannibalism could cause the periodicity in recruitment observed in Lakes Ontario and Erie (Appendix). Simulations were run using RAMAS/STAGE software (Ferson 1991). Simulations were run for a length of 50 years with various levels of cannibalism and stochasticity and can be grouped into three general categories.

(1) Simulations in which only yearlings were allowed to cannibalize YOYs. Several model configurations were run to evaluate the effects of yearling predation on YOYs under different levels of cannibalism and stochasticity.

(2) Simulations of separate adult age-classes, age-2 and older, cannibalizing YOYs. Each of these simulation configurations was run at levels of cannibalism equal to those simulated for the yearling cohort and at levels calculated from our empirical data.

(3) Simulations of combinations of age-classes, yearling and older, preying on YOYs. Age-2 and older smelt cannibalism levels were set to our empirically determined values for adults.

Yearling cannibalism, when simulated, was set to the levels necessary to invoke the observed recruitment cycles based on results of simulations in category 1.

There were 480 simulations run with the basic model setup described above and detailed in the Appendix. Additional simulations were run using the same format for both lakes and altering YOY mortality by varying yearling cannibalism rates above and below those necessary to induce recruitment cycles and by substituting the conditional mortality function (eq. 4) for natural and cannibalism-induced YOY mortality.

Production, consumption, and conversion efficiency

Bioenergetics simulations were run for smelt populations from both lakes using parameters and age-specific abundances from Lantry and Stewart (1993). We ran the models to simulate alternate years of a fivefold fluctuation in the abundance of yearling smelt for Lake Ontario and a 15-fold fluctuation for Lake Erie and compared results with simulations in Lantry and Stewart (1993). These fluctuation levels approximated the mean of observations from published data (O'Gorman et al. 1987; Henderson and Nepszy 1989). We used two recruitment scenarios, high and low YOY mortality, to get the five- and 15-fold fluctuations. To obtain yearling numbers, we varied YOY mortalities by ±2.5- and ±7.5-fold for Lakes Ontario and Erie, respectively. Then, we reduced the resulting yearling numbers by the successive mortalities (1 – survivorship) (Table 1) for each following age-class (Table 2). Because smelt live to age-5 or older in each lake, the annual age structure of each population is made up of smelt produced from both high- and low-recruitment year-classes. To simulate population age structure within any one calendar year, we switched abundances from high- and low-recruitment cohorts at ages 2 and 4. This resulted, for example, in simulated population age structure in

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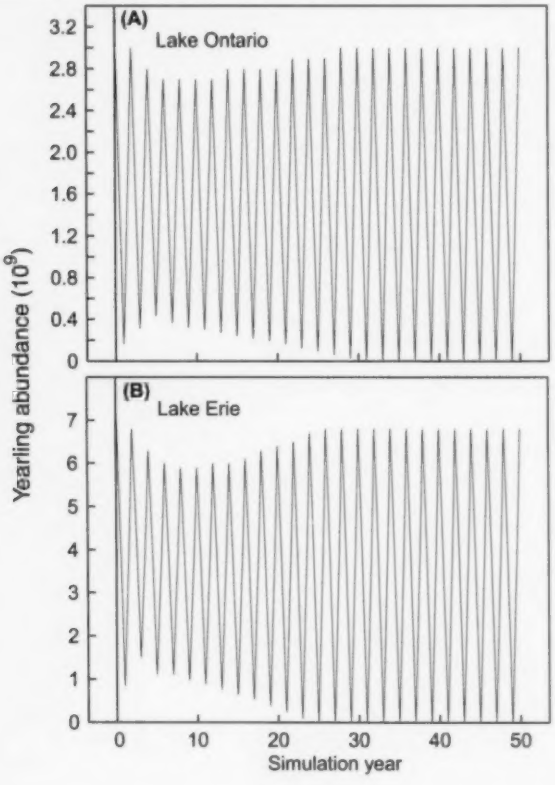
Fig. 2. Annual abundances of yearling rainbow smelt from stage-based matrix model simulations of yearling cannibalism on YOY smelt for the Lakes Ontario and Erie populations. Natural mortality and cannibalism were simulated as independent events without stochasticity. The cannibalism rates were 0.95 and 0.89 YOY per yearling for Lakes Ontario and Erie, respectively. These rates were the values necessary to perpetuate nondampening yearling abundance oscillations similar in magnitude to those observed for these lakes. Natural mortality was simulated before deaths due to cannibalism were subtracted from the YOY cohort. When natural mortality was simulated after deaths due to cannibalism were subtracted, rates of 5.8 and 3.9 YOYs per yearling for Lakes Ontario and Erie, respectively, were necessary to achieve abundance cycles similar to those shown here.



years of high YOY mortality having a high relative abundance of yearlings, low relative abundance of age 2s, high relative abundance of age 3s, and so on. Model simulations were then run to evaluate differences in production, consumption, and gross conversion efficiency between years of mean, high, and low yearling smelt abundance.

Results

Population dynamics

Simulations of yearling smelt cannibalizing YOYs (Fig. 2) were the only scenarios that brought about the 2-year cycle similar to the patterns of alternating recruitment observed from Lakes Ontario and Erie. When cannibalism was simulated as an event independent of natural mortality (eqs. 2 and 3), parameter sequence within equations was important in determining abundance at succeeding time steps. In these simulations, natural mortality was modeled as a proportion of total numbers within an age-class, while cannibalism was modeled as a distinct whole number dependent only on the number of predators and not prey. Natural mortality produced a number of "deaths" that were dependent on the YOY age-class abundance, and cannibalism produced a number of deaths without regard to the number of YOYs present. When deaths due to natural mortality were removed from the population before cannibalism in simulations (eq. 2, without stochasticity or adult cannibalism), less than one YOY eaten per yearling per year invoked alternate year cycles in yearling smelt abundance greater than those observed from Lakes Ontario and Erie (Fig. 2). When deaths due to natural mortality were removed from each population after deaths due to cannibalism mortality (eq. 3), 5.8 YOYs eaten per yearling per year in Lake Ontario and 4.9 per year in Lake Erie were necessary to invoke alternate year cycles similar to those shown in Fig. 2. Both simulation strategies produced similar mean abundances for yearling and adult smelt averaged over a 50-year simulation. Smelt population abundances produced from these simulations were below initial values. When natural mortality and cannibalism mortality were modeled as a conditional function (eq. 4) where total mortality was calculated by summing the instantaneous rates ($Z_n + Z_c$), results were the same as those from eq. 3. Levels of cannibalism greater than those necessary to induce abundance oscillations raised the amplitude of oscillations and rapidly drove the number of yearling smelt to zero in low-abundance years but did not change the population mean abundance. Cannibalism levels less than those required to induce the alternate year recruitment pattern produced abundance cycles, but those cycles dampened out to a stable mean with time and had lower amplitudes than persistent cycles. The mean abundances produced from these simulations were below initial values but above values from simulations that produced persistent abundance oscillations.

Simulations of adult smelt cannibalizing YOYs did not produce a 2-year oscillation in yearling abundance (Fig. 3). Simulation of adult cannibalism (≥age 2) produced three general effects. (*i*) The population mean abundances averaged over 50-year simulations were reduced below both initial levels and levels produced from simulations with only yearling cannibalism (Figs. 2 and 3). (*ii*) Adult cannibalism alone produced fluctuations in the abundance of yearling smelt that had greater than 2-year periods (Fig. 3). When cannibalism from one adult age-class was simulated, without yearling cannibalism or stochasticity, the period of the oscillation in yearling abundance was generally equal to twice the age of the cannibalizing age-class. The time lag between the cannibalism event upon YOYs and their eventual recruitment to the age of the cannibalizing adults determined the period of abundance cycles. When cannibalism was simulated from all adult age-classes, the period of the oscillation in yearling abundance was dependent on the abundance of each adult age-class and the level of cannibalism simulated. The year-class that exerted the strongest cannibalism pressure shifted the period of the oscillation toward a value of about twice the age of that year-class. (*iii*) When cannibalism due to both adults and yearlings was simulated together

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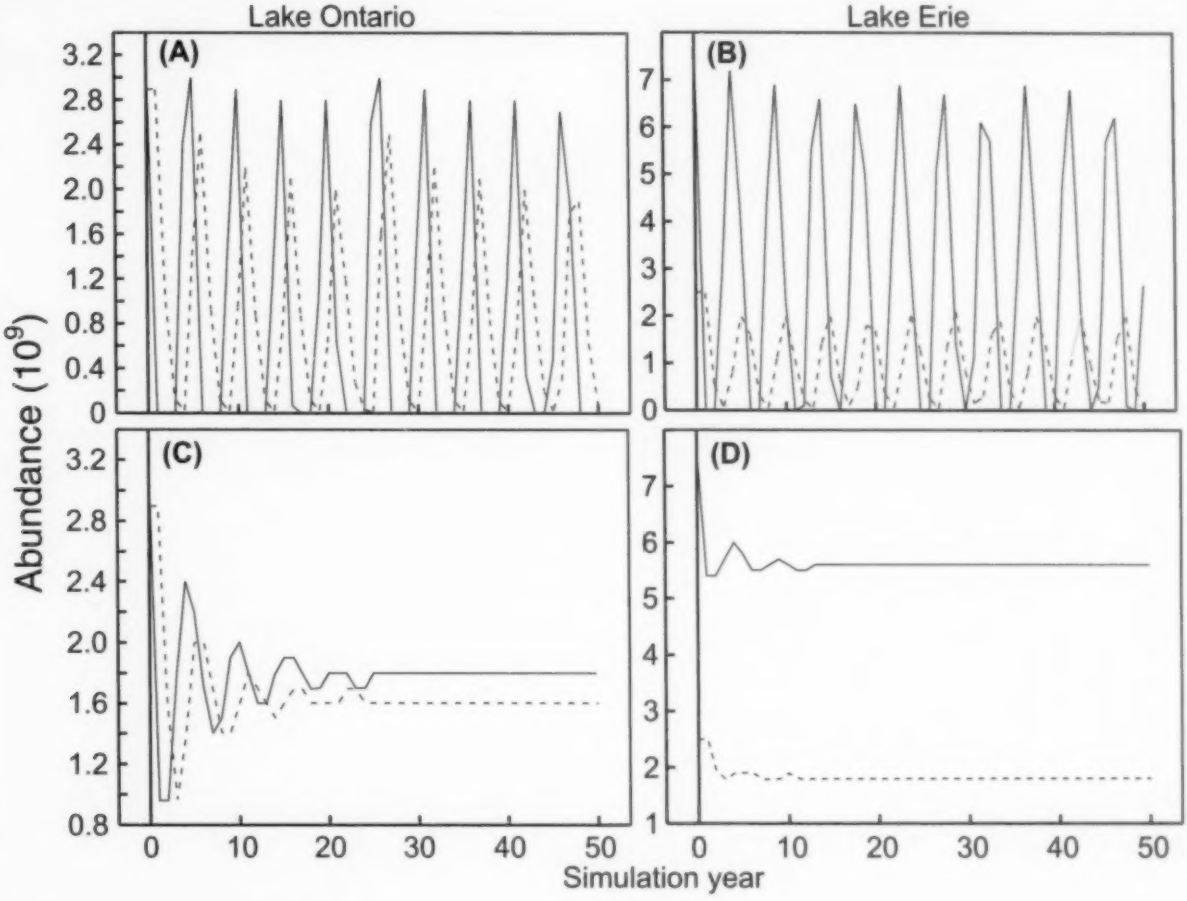
Fig. 3. Annual abundances of yearling (solid line) and adult (dotted line) rainbow smelt from stage-based matrix model simulations of adult cannibalism on YOY smelt for the Lakes Ontario and Erie populations. Natural mortality and cannibalism were simulated as independent events without stochasticity. Cannibalism rates for each adult age-class, age 2 and older, were determined from our earlier smelt diet study (Lantry 1991). Adult smelt cannibalism rates were 3.85 YOYs per age-2 individual and 6.2 YOYs per age-3 to age-6 individuals for the Lake Ontario population and 2.8 YOYs per age-2, 8.3 YOYs per age-3, 6.1 YOYs per age-4, and 0.44 YOY per age-5 individual for the Lake Erie population. Natural mortality was simulated before deaths due to cannibalism were subtracted from the YOY cohort in Figs. 3A and 3B. Natural mortality was simulated after deaths due to cannibalism were subtracted from the YOY cohort in Figs. 3C and 3D.



in the same model run, the amount of yearling cannibalism necessary to reproduce observed 2-year abundance fluctuations was greater than when only yearling cannibalism was simulated (Fig. 4).

In adult cannibalism simulations, the sequence of mortality events within the equations for YOY abundance (eqs. 2 and 3) also affected results in the same general manner as discussed for simulations of yearling cannibalism. When adult cannibalism was simulated before natural mortality (eq. 2), persistent abundance cycles were produced with a period greater than 2 years (Figs. 3A, 3B, 4A, and 4). When the same level of adult cannibalism was simulated after natural mortality (eq. 3), abundance cycles with similar periods had lower amplitudes and dampened out to a stable abundance with time (Figs. 3C, 3D, 4C, and 4D).

Simulations of stochasticity in survivorship of all age-classes disrupted the alternate year periodicity in abundance fluctuations produced when yearling smelt ate YOYs (Fig. 5). Increasing the variability in survivorship caused increases in the amplitude of abundance fluctuations. While greater variance in survivorship could produce abundance fluctuations in the yearling smelt populations similar to those observed at lower levels of cannibalism, the cycle of alternating year-class strength (2-year periodicity) was not present. Simulations that employed stochastic parameters had abundance frequencies similar to the stochastic parameter (yearling cannibalism = 5.8 YOYs per year, cannibalism stochasticity = 5%, and survivorship stochasticity = 20%) (Fig. 5). Simulations of stochastic survivorship of YOYs only (i.e., recruitment variability) did not change the amount of cannibalism by yearlings necessary to invoke abundance oscillations similar to those observed from the literature.

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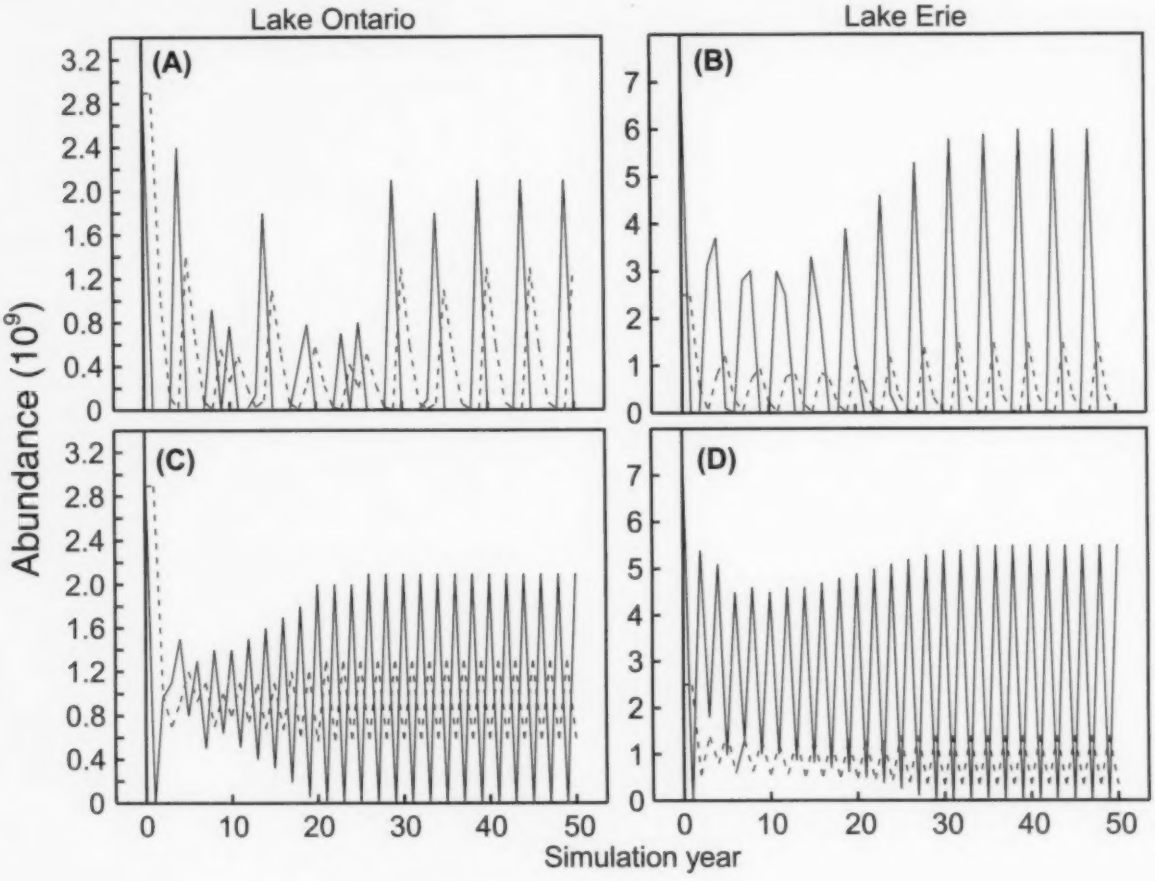
Fig. 4. Annual abundances of yearling (solid line) and adult (dotted line) rainbow smelt from stage-based matrix model simulations of yearling and adult cannibalism on YOY smelt for the Lakes Ontario and Erie populations. Natural mortality and cannibalism were simulated as independent events without stochasticity. Cannibalism rates for each adult age-class, age 2 and older, were determined from our earlier smelt diet study (Lantry 1991). Individual adult smelt cannibalism rates were the same as those in Fig. 3. Natural mortality was simulated before deaths due to cannibalism were subtracted from the YOY cohort in Figs. 4A and 4B. For these simulations, cannibalism rates of 3.5 and 1.1 YOYs per yearling for Lakes Ontario and Erie, respectively, were necessary to induce abundance oscillations similar to those observed. Natural mortality was simulated after deaths due to cannibalism are subtracted from the YOY cohort in Figs. 4C and 4D. For these simulations, cannibalism rates of 7.0 and 4.0 YOYs per yearling for Lakes Ontario and Erie, respectively, were necessary to invoke similar abundance oscillations.



Use of the Beverton and Holt (1957) density-dependence function kept YOY abundances relatively stable and tended to minimize the effect of survivorship stochasticity on YOY abundance.

Production, consumption, and conversion efficiency

Bioenergetics simulations of alternating recruitment (Table 3) in Lake Ontario produced variations of about 6–8% in gross production around the nominal simulation value of 3.600×10^{10} g, while total annual consumption varied by less than 2% from the nominal value of 2.317×10^{11} g. As a result, gross conversion efficiency varied by only about 7% between simulations with high or low YOY survivorship and the nominal simulation value of 14.4%. Simulations for Lake Erie smelt populations yielded much more dramatic results. Population gross production values produced in simulations of high and low YOY survivorship varied by about 30–60% around the nominal simulation value of 1.350×10^{11} g. Year-to-year differences in total annual consumption from simulations of high and low YOY survivorship varied by about 21–35% around the nominal value of 6.415×10^{11} g. Hence, resulting gross conversion efficiencies varied by about 12–18% around the nominal simulation value of 21%. The importance of population age structure was emphasized in Lake Erie simulations when the biomass of spawning-aged smelt changed by 2.4-fold between years of high and low YOY survivorship. However, smaller recruitment oscillations, greater abundance of age-3 and older

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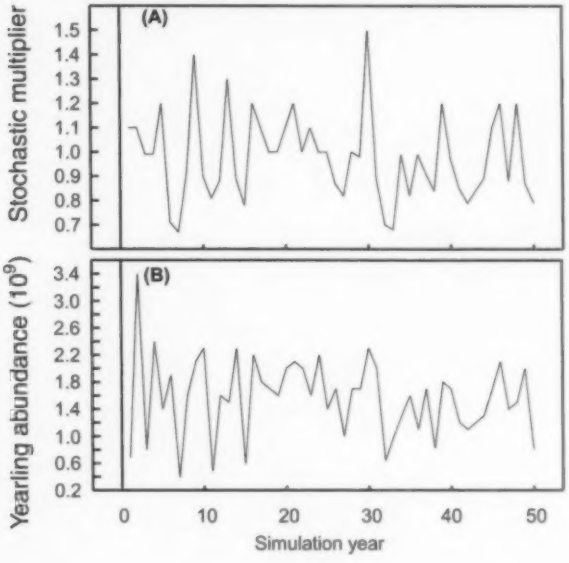
Fig. 5. Pattern similarities between the values of the stochastic multiplier for the survivorship coefficients and the subsequent simulated abundance of yearling rainbow smelt. Figure 5A shows the pattern produced in the stochastic multiplier (mean of 1.0 and variance of 0.0299) when an approximately 20% variation in the survivorship coefficients is simulated. Figure 5B is the abundance of yearling smelt from simulations of yearlings cannibalizing 5.8 YOYs each and having 5 and 20% stochasticity in the cannibalism and survivorship parameters, respectively.



smelt, and the extended age-class structure maintained the biomass of spawners at a relatively constant level for the Lake Ontario population.

Discussion

Our results support the hypothesis that cannibalism by yearling rainbow smelt upon YOYs drives the alternating pattern of strong and weak years of recruitment in Lakes Ontario and Erie. The 2-year cycle of alternating high and low yearling abundance was reproduced only in simulations of yearling cannibalism on YOYs. Our simulations of adult cannibalism (≥age 2) did produce cyclic population behavior, but all resulting abundance oscillations had periods greater than 2 years. When we simulated stochasticity in survivorship (CV = 5–20%), with and without the presence of cannibalism, population cycles became irregular, the period of oscillations fluctuated, and periods were generally greater than 2 years.

Certain questions need to be addressed to further support the cannibalism hypothesis. Do yearling and YOY spatial distributions overlap, providing the opportunity for cannibalism? Do yearling smelt commonly consume larval smelt in the presence of alternative prey? Are the relatively low individual rates of cannibalism observed in yearling smelt enough to bring about the cyclic recruitment patterns observed? And can other possible causes of recruitment cycles be rejected?

Spatial overlap of predator and prey is necessary for predation to occur. Observations indicate that young (YOY and yearling) and adult rainbow smelt in the Great Lakes segregate according to temperature, with the greatest separation during times of thermal stratification. When the lakes are stratified, most of the large adults are located along the bottom below the thermocline in temperatures from 6 to 12°C (Dryer 1966; Wells 1968). Yearling smelt are often observed in bottom trawl catches just above the thermocline (Ferguson 1965; Dryer 1966). Larvae in Lake Michigan from May to August disperse through the water column to depths of at least 15 m, with substantial numbers caught in temperatures of 10–16°C (Tin and Jude 1983). During late summer, juveniles (age 0) concentrate along the bottom above the thermocline (Ferguson 1965; Tin and Jude 1983). The distributions of young and adult smelt begin to overlap in the fall when stratification breaks down and the lakes become homeothermic (Ferguson 1965; Wells 1968). The opportunity for spatial overlap between yearling and YOY smelt in Lakes Ontario and Erie occurs throughout the year, while the opportunity for spatial overlap between YOYs and adults only increases substantially after stratification breaks down in the fall.

Smelt have been described in the literature as voracious predators, and field and laboratory evidence demonstrates that yearling smelt eat larval fish (Evans and Loftus 1987; Henderson and Nepszy 1989; Luecke et al. 1990). Yearling and adult smelt include YOY smelt in their diets, and both field and laboratory evidence indicates that yearling smelt predation on fish larvae continues in the presence of alternative prey (Chen 1972; Luecke et al. 1990; Lantry 1991; He and LaBar 1994). While past observations (e.g., Evans and Waring 1987; Henderson and Nepszy 1989) have suggested that cannibalism by yearling smelt drives alternating year-class cycles, few larvae have been observed from stomachs. Henderson and Nepszy (1989) found that only 2% of yearling smelt stomachs sampled from Lake Erie between May 10 and August 25, 1976, contained YOY smelt. Larval fish are, however, digested rapidly and may go undetected in stomach analyses. Evidence suggests that digestion by alewife (*Alosa pseudoharengus*) may render smelt larvae unrecognizable in less than 1 h, and lake herring (*Coregonus artedi*) embryos consumed by smelt at 11°C are unrecognizable within 2 h (Crowder 1980). Knowing that digestion of fish larvae proceeds swiftly, then it is reasonable to expect that cannibalization rates in smelt populations may be substantially greater than observations from stomach analysis would indicate. By using age-specific smelt abundances and daily mortality rates from Lantry and Stewart (1993) and assuming that yearling smelt were cannibalizing YOYs on a daily basis, we calculated the time needed to force the abundance oscillations observed in both Lakes Ontario and Erie (one YOY eaten per yearling per day; cannibalism deaths subtracted out after natural mortality). We found that at the 2% cannibalism level observed by Henderson and Nepszy (1989), yearling smelt could drive the 15-fold abundance fluctuations observed in Lake Erie within 59 days after June 1. For the Lake Ontario population, about 5.1% of the yearling

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Table 3. Simulated total population production, consumption, and biomass for bioenergetics models of alternate year-class strength of rainbow smelt in Lake Ontario and Lake Erie.

	Total annual gross production ($\times 10^{10}$ g)	Total annual net production ($\times 10^{10}$ g)	Total annual consumption ($\times 10^{11}$ g)	Mean monthly biomass ($\times 10^{10}$ g)	Gross conversion efficiency (g/g)	Net conversion efficiency (g/g)	*P/B* (g/g)
Lake Ontario							
Nominal	3.600	3.312	2.317	3.331	15.5	14.3	1.09
Low	3.295 (–8%)	3.007 (–9%)	2.284 (–1%)	3.253 (–2%)	14.4 (–7%)	13.2 (–8%)	1.16 (7%)
High	3.806 (6%)	3.525 (6%)	2.278 (2%)	3.317 (<1%)	16.7 (8%)	15.5 (8%)	1.06 (–2%)
Lake Erie							
Nominal	13.50	12.80	6.415	8.790	21.0	20.0	1.54
Low	21.47 (59%)	20.28 (58%)	8.677 (35%)	11.31 (29%)	24.7 (18%)	23.4 (17%)	1.90 (24%)
High	92.71 (–31%)	8.843 (–31%)	5.080 (–21%)	7.573 (–14%)	18.2 (–13%)	17.4 (–13%)	1.22 (–20%)

Note: "Nominal" simulations use values from Lantry and Stewart (1993) representing mean population abundances and survivorships, "low" simulations are for years with low YOY survivorship (Lake Ontario = 10.3%, Lake Erie = 3.1%), and "high" simulations are for years with high YOY survivorship (Lake Ontario = 51.3%, Lake Erie = 46.9%). *P/B* is production to mean monthly biomass ratio. Values in parentheses are percentage differences from nominal simulation values.

fish had to consume one YOY on a daily basis between the time larval smelt first became pelagic and the time they became completely transformed into juveniles (about 68 days, June 1 to August 8) to cause the sevenfold abundance fluctuations observed. Our cannibalism calculations and simulation results indicate that it is highly likely that cannibalism is driving the yearling smelt abundance fluctuation in both lakes. It is also important to note that from our calculations, very reasonable levels of cannibalism occurring only at the time when larval smelt are most susceptible to yearling predation (before transformation) will induce recruitment oscillations equal in amplitude to those observed.

Interactions other than cannibalism have also led to alternating recruitment patterns in fish populations. Alternating recruitment cycles have been observed in walleye populations in northern Ontario, Canada (Ritchie and Colby 1988), roach (*Rutilus rutilus*) populations in Alferden Broad, England (Cryer et al. 1986), vendace (*Coregonus albula*) populations in Finland and Sweden (Hamrin and Persson 1986; Auvinen 1994), and longfin smelt (*Spirinchus thaleichthys*) populations in Lake Washington, U.S.A. (Moulton 1974).

Cycles in predator abundance are often linked to abundance cycles in their prey (Krebs 1991). Alternating recruitment of walleye in Savanne Lake, Ontario, is linked to the 2-year life cycles in burrowing mayflies (*Hexagenia limbata*), whose production and density are greater in even years (Ritchie and Colby 1988). Mayflies, consumed by walleye from January to June, buffer cannibalism on YOY walleye and enhance fecundity in adult walleye. While it is possible, we found no observations from the literature on Great Lakes smelt diet and life history that link cyclical prey abundance to smelt abundance (for a list of smelt diet and life history citations, see Lantry 1991; Lantry and Stewart 1993).

Recruitment variability in fish populations is also linked to life span, age at maturity, and size-related reproductive output (Wooton 1990). In instances where alternating recruitment is observed in fish populations with abbreviated life spans (≤2 years), intraspecific competition is often cited as the cause. Intraspecific competition for planktonic food resources within the roach and vendance populations is thought to drive their recruitment oscillations, while population age structure is important in maintaining the 2-year periodicity of the cycles (Cryer et al. 1986; Hamrin and Persson 1986; Auvinen 1994).

During the periods when alternating recruitment cycles were observed in Lakes Ontario and Erie, multiple spawning age-classes have been present, reducing the chances that intraspecific competition was the cause of the cycles. In comparison with Lake Erie, however, the Lake Ontario smelt population had an extended age-class structure and has experienced abundance oscillations with lower relative amplitudes. As a result, the simulated per capita cannibalism level by yearling smelt necessary to invoke sevenfold recruitment oscillations in Lake Ontario is over three times greater than that necessary to force 15-fold oscillations for the Lake Erie population.

Alternating year-class strength in rainbow smelt in other systems has been attributed to competition. The smelt population in Lake Simcoe, Ontario, exhibits alternating year-class strength that is thought to be caused by competition between YOY and yearling smelt which have similar diets consisting primarily of zooplankton (Evans and Waring 1987). Earlier literature reviews and diet studies of Great Lakes smelt (Lantry 1991; Urban and Brandt 1993) indicate that YOY smelt (<60 mm) mainly consume zooplankton during their first summer and begin to include benthic invertebrates in the fall, while yearling diets include large proportions of benthic invertebrates throughout the year. Differences in diets in addition to the presence of more than one spawning age-class in Lakes Ontario and Erie lessen the likelihood that competition alone was the cause of recruitment cycles.

He and LaBar (1994) observed 3.5-fold variation in the catch per unit effort of smelt taken in midwater trawls from 1984 to 1992 in Lake Champlain. Their observations indicate that both adults and yearling smelt cannibalize YOYs. In their model of the effect of predation and cannibalism on that smelt population, they found cannibalism to be the most important factor in determining long-term mean abundances of smelt. The absence of alternating recruitment in Lake Champlain smelt stocks, however, may be attributable to both extended age-class structure and high stock biomass. When He and LaBar (1994) simulated cannibalism by age 1–5 smelt on both YOY and yearlings, the period of abun-

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dance oscillations was 4 years. When they constrained their simulations to cannibalism by age-2 fish, a 2-year periodicity was produced. In our simulations constraining cannibalism to age-2 smelt eating YOYs, a 4-year periodicity was produced. When we simulated adult cannibalism on yearlings, we could not produce a 2-year cycle. The 2-year pattern that He and LaBar (1994) simulated may have been dependent on their stochastic generator.

Alternating recruitment cycles for fish populations often depend on reduced age structure with few spawning age-classes (Cryer et al. 1986; Hamrin and Persson 1986; Auvinen 1994). Heavy demands on smelt populations from commercial fishing in Lake Erie and from predation by sport fish stocks in both Lakes Erie and Ontario may have facilitated alternating recruitment cycles. Age-class structure and size-at-age in both lakes and population abundance in Lake Erie were declining during the period covered by this study (Christie et al. 1987; Henderson and Nepszy 1989). Alternate-year recruitment cycles in Lake Erie smelt populations appeared about 1960 when a developing commercial fishery for smelt had begun to drive their total abundance down (Henderson and Nepszy 1989). Data on alternate-year recruitment patterns in Lake Ontario go back only to 1978 (O'Gorman et al. 1987; Jones et al. 1993). Earlier age composition data, however, indicate that after 1976, the proportion of the adult smelt population in Lake Ontario at age 2 shifted from 20.7 to 46.3% in 1977 and up to over 56% in 1981 (Christie et al. 1987). This shift in age-class structure to a population dominated by yearling and age-2 smelt was apparently due to lake trout (*Salvelinus namaycush*) predation and occurred as stocking of trout and salmon was increasing sharply from about 2.8 million fish in 1974 to a peak of over 8 million in 1986.

Cyclic abundance patterns have important implications for the efficiency of trophic transfer. Alternating recruitment cycles in Lake Erie can produce 30–60% fluctuations in total annual production and 12–18% fluctuations in population gross conversion efficiency for rainbow smelt. Variations in production and conversion efficiency were much less in Lake Ontario where age-class structure buffered some effects. These variations in production can then be transferred to predators. Oscillations in recruitment strength of the longfin smelt population in Lake Washington directly affected rainbow trout diets. Beauchamp (1990) found that during winters of low longfin smelt abundance, the apparent overwinter ration of rainbow trout that fed upon them was reduced by about 50%.

Using our model, we were able to simulate the maximum observed abundance fluctuations from Lakes Ontario and Erie at the low levels of cannibalism observed in the field. Like smelt cannibalism in the Great Lakes, there are many instances where recruitment variability and fish stock declines have been thought to be due to predation (e.g., Loftus and Hulsman 1986; Luecke et al. 1990; Mason and Brandt 1996). Empirical evidence from fish stomachs is often not sufficient to support the hypothesis that predation on larvae is causing this recruitment variability. Our analysis and others like it, however, should prove beneficial in teasing apart the predation interactions that impact recruitment. Two commonly encountered, but difficult to sample, predation situations arise when (*i*) predation on larvae occurs at high levels over a short period, making sample timing critical (Mason and Brandt 1996), and (*ii*) predation occurs at practically undetectable levels over an extended period, making the number of larvae consumed seem inconsequential (e.g., this study). Each situation has in the past led to conflicting views on the mechanisms forcing recruitment variability (Evans and Loftus 1987; Henderson and Nepszy 1989; Mason and Brandt 1996). Our analysis, however, indicates that when observed predation levels are expanded to include all available predators feeding throughout the time window of prey vulnerability, the observed levels are often sufficient to completely eliminate whole year-classes of the prey.

While a negative interaction between yearling and YOY smelt seems to induce alternating recruitment cycles in Great Lakes smelt stocks, an abbreviated age-class structure seems to be a prerequisite or, at least, can amplify the effect. Age-class structures like those that precipitated extreme recruitment variability and year-class failure in Great Lakes smelt stocks and European vendace and roach stocks now threaten to induce similar effects in many fish populations (e.g., Great Lakes alewife and yellow perch (*Perca flavescens*) populations). Hence, management decisions regarding fisheries experiencing substantial exploitation or heavy predation pressure (e.g., size-selective predation from high densities of stocked predators; Rand et al. 1994) will need to consider the possibility of recruitment variation brought on by a reduced age-class structure.

Our modeling analysis lends strong support to the cannibalism hypothesis. This evaluation indicates that density-dependent compensatory mechanisms like cannibalism may turn out to be destabilizing interactions at low biomass and force dramatic cycles in abundance. In the absence of suitable alternative prey, radically different year-to-year levels of total annual production brought on by recruitment cycles in prey fish stocks can impact predators dependent on them. The amount of piscivory that these prey stocks can maintain in years of low production may set the upper limits of predator biomass when few alternative prey are available. Regardless of whether prey stocks are exploited by predatory fish or commercial fisheries, there will be benefits from managing for a more complete age structure in the prey fish.

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Appendix. Types of matrix model simulations for rainbow smelt from Lakes Ontario and Erie.

		Stochasticity		
Cannibalistic cohort	Cannibalism level (no. YOYs/fish)	Survivorship	Cannibalism	No. simulations
Lake Ontario				
Yearling	0A, 0B, 0.95A, 5.8B	Invariant	Invariant	4
Yearling	0A, 0B, 0.95A, 5.8B	Invariant	5%	4
Yearling	0A, 0B, 0.95A, 5.8B	5%	0%	4
Yearling	0A, 0B, 0.95A, 5.8B	10%	0%	4
Yearling	0A, 0B, 0.95A, 5.8B	20%	0%	4
Yearling	0A, 0B, 0.95A, 5.8B	5%	5%	4
Yearling	0A, 0B, 0.95A, 5.8B	10%	5%	4
Yearling	0A, 0B, 0.95A, 5.8B	20%	5%	4
Ages 2–6[a]	0.95A, 5.8B, empirical A and B	Invariant	Invariant	20
Ages 2–6[a]	0.95A, 5.8B, empirical A and B	Invariant	5%	20
Ages 2–6[a]	0.95A, 5.8B, empirical A and B	5%	0%	20
Ages 2–6[a]	0.95A, 5.8B, empirical A and B	10%	0%	20
Ages 2–6[a]	0.95A, 5.8B, empirical A and B	20%	0%	20
Ages 2–6[a]	0.95A, 5.8B, empirical A and B	5%	5%	20
Ages 2–6[a]	0.95A, 5.8B, empirical A and B	10%	5%	20
Ages 2–6[a]	0.95A, 5.8B, empirical A and B	20%	5%	20
All, 2–6, 3–6, 4–6	3.5A,[b] 7B,[b] empirical A and B	Invariant	Invariant	8
All, 2–6, 3–6, 4–6	3.5A,[b] 7B,[b] empirical A and B	Invariant	5%	8
All, 2–6, 3–6, 4–6	3.5A,[b] 7B,[b] empirical A and B	5%	0%	8
All, 2–6, 3–6, 4–6	3.5A,[b] 7B,[b] empirical A and B	10%	0%	8
All, 2–6, 3–6, 4–6	3.5A,[b] 7B,[b] empirical A and B	20%	0%	8
All, 2–6, 3–6, 4–6	3.5A,[b] 7B,[b] empirical A and B	5%	5%	8
All, 2–6, 3–6, 4–6	3.5A,[b] 7B,[b] empirical A and B	10%	5%	8
All, 2–6, 3–6, 4–6	03.5A,[b] 7B,[b] empirical A and B	20%	5%	8
Lake Erie				
Yearling	0A, 0B, 0.89A, 4.9B	Invariant	Invariant	4
Yearling	0A, 0B, 0.89A, 4.9B	Invariant	5%	4
Yearling	0A, 0B, 0.89A, 4.9B	5%	0%	4
Yearling	0A, 0B, 0.89A, 4.9B	10%	0%	4
Yearling	0A, 0B, 0.89A, 4.9B	20%	0%	4
Yearling	0A, 0B, 0.89A, 4.9B	5%	5%	4
Yearling	0A, 0B, 0.89A, 4.9B	10%	5%	4
Yearling	0A, 0B, 0.89A, 4.9B	20%	5%	4
Ages 2–5[a]	0.89A, 4.9B, empirical A and B	Invariant	Invariant	16
Ages 2–5[a]	0.89A, 4.9B, empirical A and B	Invariant	5%	16
Ages 2–5[a]	0.89A, 4.9B, empirical A and B	5%	0%	16
Ages 2–5[a]	0.89A, 4.9B, empirical A and B	10%	0%	16
Ages 2–5[a]	0.89A, 4.9B, empirical A and B	20%	0%	16
Ages 2–5[a]	0.89A, 4.9B, empirical A and B	5%	5%	16
Ages 2–5[a]	0.89A, 4.9B, empirical A and B	10%	5%	16
Ages 2–5[a]	0.89A, 4.9B, empirical A and B	20%	5%	16
All, 2–5, 3–5, 4–5	1.1A,[b] 4B,[b] empirical A and B	Invariant	Invariant	8
All, 2–5, 3–5, 4–5	1.1A,[b] 4B,[b] empirical A and B	Invariant	5%	8
All, 2–5, 3–5, 4–5	1.1A,[b] 4B,[b] empirical A and B	5%	0%	8

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Appendix (*concluded*).

Cannibalistic cohort	Cannibalism level (no. YOYs/fish)	Stochasticity		No. simulations
		Survivorship	Cannibalism	
All, 2–5, 3–5, 4–5	1.1A,[b] 4B,[b] empirical A and B	10%	0%	8
All, 2–5, 3–5, 4–5	1.1A,[b] 4B,[b] empirical A and B	20%	0%	8
All, 2–5, 3–5, 4–5	1.1A,[b] 4B,[b] empirical A and B	5%	5%	8
All, 2–5, 3–5, 4–5	1.1A,[b] 4B,[b] empirical A and B	10%	5%	8
All, 2–5, 3–5, 4–5	1.1A,[b] 4B,[b] empirical A and B	20%	5%	8

Note: The levels of cannibalism are the number of YOYs eaten per individual annually. Each cannibalism level was simulated separately for each model configuration. The cannibalistic cohort was the age-class(s) within the model configuration that was preying on YOY smelt. "A" denotes that the indicated level of cannibalism was subtracted out of the YOY cohort after natural mortality losses were subtracted. "B" denotes that the indicated level of cannibalism was subtracted out of the YOY cohort before natural mortality losses were subtracted. Stochasticity values in the survivorship and cannibalism parameters are percentages of nominal parameter values. Survivorship and cannibalism stochasticity values listed were applied to all cohorts in each simulation. "Empirical" is the amount of YOY smelt eaten per adult smelt and was calculated from diet data on Lake Michigan smelt (Lantry, B.L. 1991. Ecological energetics of rainbow smelt in the Laurentian Great Lakes: an interlake comparison. M.S. thesis, College of Environmental Science and Forestry, State University of New York, Syracuse, N.Y.) where 2% of age-2 and 6% of age-3 and older smelt were cannibalizing YOYs. For Lake Ontario, "empirical" represents 3.85 YOYs per age-2 smelt and 6.2 YOYs per age-3 and older smelt. For Lake Erie, "empirical" represents 2.8 YOYs per age-2 smelt, 8.3 YOYs per age-3 smelt, 6.1 YOYs per age-4 smelt, and 0.44 YOYs per age-5 smelt. Additional simulations were run using the same format below for both lakes and altering YOY mortality by varying yearling cannibalism rates above and below those necessary to induce recruitment cycles and by substituting the conditional mortality function (eq. 4) for natural and cannibalism-induced YOY mortality.

[a]Denotes that each configuration was run with the indicated levels of cannibalism and stochasticity and with only one of the listed age-classes preying on YOY smelt until all age-classes were simulated preying on YOY smelt.

[b]Denotes that these values are only used to simulate coefficients for the yearling cohort in simulations where all cohorts yearling and older are preying on YOY smelt.

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Cross-tributary analysis of parr to smolt recruitment of Atlantic salmon (*Salmo salar*)

Kevin G. Whalen, Donna L. Parrish, Martha E. Mather, and James R. McMenemy

Abstract: We used estimates of Atlantic salmon (*Salmo salar*) parr and smolt density, estimated in three tributaries of the West River, Vermont, U.S.A., to determine (*i*) if smolt recruitment is density dependent or independent of parr density, (*ii*) if the proportion of parr migrating as smolts and cohort survival differ among tributaries, and (*iii*) the effect of parr maturity on smolt production and recruitment variability. We found that parr to smolt recruitment was best described with a linear function providing no evidence for density dependence in the recruitment dynamics of parr and smolts at the tributary scale. The proportion of age-1 parr recruiting to age-2 smolts did not systematically differ among tributaries or years (overall mean ± 95% CL: 18 ± 11%, range = 9–37%), and mean age-1 to age-2 survival ranged less than twofold among tributaries (27–46%) and was independent of cohort density. Survival of age-1 mature (39%) and immature (33%) parr was similar, but probability of smolting for mature parr (0.21) was threefold less than for immature parr (0.76). Quantifying smolt recruitment pathways involving parr maturation helped elucidate the population-level effect of parr maturation on smolt production and recruitment variability.

Résumé : Nous nous sommes servis d'estimations de la densité des tacons et des smolts de saumon atlantique (*Salmo salar*), établies pour trois affluents de la rivière West, Vermont (É.-U.), pour déterminer (*i*) si le recrutement des smolts est dépendant ou indépendant de la densité des tacons; (*ii*) si la proportion des tacons qui migrent à l'état de smolt et la survie des cohortes diffèrent d'un affluent à l'autre; et, (*iii*) l'effet de la maturité des tacons sur la variabilité de la production et du recrutement des smolts. Nous avons établi que le recrutement du tacon au smolt trouvait sa meilleure description dans une fonction linéaire qui ne fournissait aucune indication d'une dépendance à l'égard de la densité dans la dynamique de recrutement des tacons et des smolts à l'échelle de l'affluent. La proportion des tacons d'âge 1 recrutant au stade de smolt d'âge 2 ne différait pas systématiquement d'un affluent à l'autre ni d'une année à l'autre (moyenne générale ± 95% LC : 18% ± 11; fourchette = 9–37%), et la survie moyenne de l'âge 1 à l'âge 2 allait à peine du simple au double parmi les affluents (27 à 46%) et était indépendante de la densité des cohortes. La survie des tacons d'âge 1 matures (39%) et immatures (33%) était similaire, mais la probabilité de smoltification pour les tacons matures (0,21) était trois fois moins élevée que pour les tacons immatures (0,76). La quantification des voies de recrutement des smolts passant par la maturation des tacons a aidé à mieux comprendre l'effet au niveau de la population de la maturation des tacons sur la variabilité de la production et du recrutement des smolts.

[Traduit par la Rédaction]

Introduction

The management, conservation, and restoration of Atlantic salmon (*Salmo salar*) populations necessitate a full understanding of recruitment processes throughout the life cycle. A recruitment step of primary importance to populations is the ontogenetic shift of parr from freshwater or estuarine rearing habitat to the ocean as seawater-tolerant smolts. Estimates of smolt production are needed to determine stock–recruitment relationships for defining conservation spawning targets where salmon reproduce naturally (Chaput et al. 1998) and to determine fry and parr stocking

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K.G. Whalen.[1] Department of Forestry and Wildlife Management, Holdsworth Natural Resources Center, University of Massachusetts, Amherst, MA 01003, U.S.A.
D.L. Parrish. Vermont Cooperative Fish and Wildlife Research Unit, School of Natural Resources, University of Vermont, Burlington, VT 05405, U.S.A.[2]
M.E. Mather. Massachusetts Cooperative Fish and Wildlife Research Unit, Department of Forestry and Wildlife Management, Holdsworth Natural Resources Center, University of Massachusetts, Amherst, MA 01003, U.S.A.[2]
J.R. McMenemy. Vermont Department of Fish and Wildlife, Agency of Natural Resources, 100 Mineral Street, Suite 302, Springfield, VT 05156, U.S.A.

[1]Author to whom all correspondence should be sent at the following address: Federal Energy Regulatory Commission, Office of Energy Projects, 888 First Street NE, Washington, DC 20426, U.S.A. e-mail: kevin.whalen@ferc.fed.us
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densities maximizing survival and recruitment where populations are being restored (Whalen and LaBar 1994; McMenemy 1995). However, few studies addressing the population dynamics of juvenile Atlantic salmon have the parr to smolt transition as a focus (Elson 1975; Cunjak and Therrien 1998). Understanding the recruitment dynamics of the parr to smolt transformation is essential for the effective management and conservation of Atlantic salmon populations.

Parr to smolt recruitment dynamics may be strongly influenced by winter mortality (Orciari et al. 1994; Cunjak and Therrien 1998) and parr maturity (Myers 1984; Whalen and Parrish 1999). In winter, fluvial Atlantic salmon parr select only a portion of available habitats (Cunjak 1988; Whalen et al. 1999*a*), which may be altered by ice and flow (Cunjak and Randall 1993; Whalen et al. 1999*a*). Thus, environmental conditions in rivers and the amount of suitable winter habitat may cause smolt recruitment to be density dependent or independent depending on the density of parr. Within the framework of habitat–density relationships, sexually maturing male parr have a significantly lower probability of smolt transformation than nonmaturing parr (Myers 1984; Whalen and Parrish 1999). For Atlantic salmon, little information exists on how parr density relates to smolt density and how survival may vary among catchments supporting differing densities of parr. Little is also known specifically to what extent parr maturation may influence recruitment variability of smolts. To date, the population dynamics of the parr to smolt transition have not been studied along with life history features that may influence smolt recruitment.

When parr to smolt recruitment parameters are defined, model development can proceed to estimate smolt production from parr abundance data where it is not logistically feasible to collect smolts. Most studies of smolt recruitment have focused on one location over time and have provided information on interannual variability in recruitment (e.g., Buck and Hay 1984; Cunjak and Therrien 1998). For smolt production modeling, however, it is also necessary to understand spatial variability in parr to smolt recruitment. Smolt production models for management would have limited utility if recruitment parameters from one river were not representative of the magnitude or variability of recruitment in other rivers. Determining how parr to smolt recruitment varies across tributaries over the same time frame would therefore fill an important information gap and is needed to develop basin-level estimates of smolt production.

In this paper, we analyze parr to smolt recruitment of Atlantic salmon stocked as fry in three tributaries of the West River, Vermont, U.S.A. Herein, we refer to the transition probabilities associated with parr to smolt survival and parr to smolt transformation as smolt "recruitment," whereas we use the term smolt "production" to indicate the absolute numbers of smolts produced. Fry are stocked in Vermont tributaries of the Connecticut River as part of the effort to restore Atlantic salmon populations. All juvenile salmon, mostly unfed fry, are stocked annually in April and May in the West River and many other Connecticut River tributaries at similar densities (about 25–50·100 m^{-2}). We address the following questions. (*i*) Is smolt recruitment density dependent or independent of parr density? (*ii*) Does smolt recruitment differ among tributaries and years? (*iii*) Does survival the winter before the parr to smolt transition differ between parr becoming smolts and those remaining as parr? (*iv*) Does parr maturity affect both survival and probability of smolting? (*v*) Does parr maturation contribute most to variation in smolt recruitment relative to other factors?

Materials and methods

Study area

We studied parr to smolt recruitment from 1995 to 1997 in three tributaries of the West River: Rock River, Wardsboro Branch, and Utley Brook. Based on stream habitat surveys by the Vermont Department of Fish and Wildlife (VTFW) and the United States Forest Service (USFS), the Rock River, Wardsboro Branch, and Utley Brook are dominated by riffle habitat. Salmon populations in these tributaries are maintained by stocking primarily unfed fry at densities of 25–50 fry·100 m^{-2} (McMenemy 1995). Further details of fry stocking as well as information on the physical characteristics of these tributaries are given elsewhere (Gries et al. 1997; Whalen and Parrish 1999). The number of 100-m^2 habitat units stocked with fry ranged between 2559 and 2817 in the Rock River, 1916 and 2187 in Wardsboro Branch, and 1815 and 2106 in Utley Brook. Maintaining fry stocking densities at consistently low levels during the course of the study minimized any potential effects of variation in numbers of habitat units stocked among years. Few adult salmon enter the West River annually, and thus, the majority of smolts arise from stocked fry.

Population recruitment model

We present a conceptual model for parr to smolt recruitment in Atlantic salmon that depicts recruitment for a population having both age-2 and age-3 smolts and few individuals remaining in freshwater past age 3 (Fig. 1*a*). The model has both a main recruitment pathway (Fig. 1*a*) and a pathway involving parr maturity (Fig. 1*b*); model parameters are described in Table 1. In the first section of this paper, we focus on defining the main recruitment probabilities and address the definition of parr maturity recruitment pathways below.

For main recruitment, survival probabilities may be defined as (*i*) *SS*2 = age-2 smolts/age-1 parr, (*ii*) *SP*2 = age-2 parr/age-1 parr, and (*iii*) *SS*3 = age-3 smolts/age-2 parr. The sum of *SS*2 and *SP*2 equals survival of cohorts from age 1 to age 2, and *SS*3 equals survival of cohorts from age 2 to age 3. Survival probabilities may be determined directly when density estimates of age-1 parr, age-2 smolts, age-2 parr, and age-3 smolts are known. Below, we describe the methods used to determine parr and smolt densities.

Age-1 and age-2 parr

Parr densities were determined at multiple locations selected as representative habitat in the three study tributaries by VTFW and USFS in August and September. From 1993 to 1995, eight index stations were sampled in the Rock system and five index stations in both the Wardsboro and Utley systems. The sampling of multiple sites within each tributary and their spatial stratification minimize, as much as possible, bias associated with the extrapolation of point density estimates to areas not sampled (Crozier and Kennedy 1995). Both VTFW and USFS used a two- or three-pass removal method to estimate parr population density at each site (McMenemy 1995); scales collected from parr were used to determine age. All density estimates were standardized to the wetted streambed area based on stream habitat surveys conducted by VTFW and USFS.

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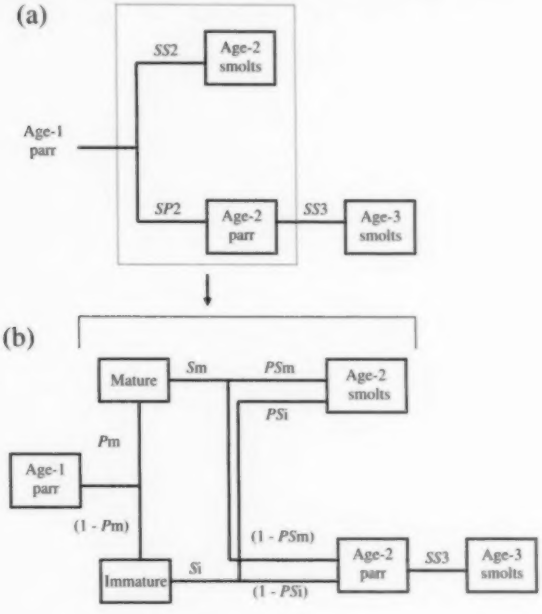
Fig. 1. Schematic representation of the Atlantic salmon age-1 to age-3 population recruitment model for West River, Vermont, tributaries for the (*a*) main recruitment and (*b*) age-1 to age-2 maturation pathways. Parameters are defined in Table 1.



Age-2 and age-3 smolts

Smolt collection

Smolt collection procedures and gear are described in Whalen et al. (1999*b*). Briefly, smolts were collected with net weirs in 1994 and with counting fences in 1995. Sampling was attempted in 1996, but heavy flooding precluded the estimation of smolt population size. Net weirs had 1-cm-mesh net wings of varying length and counting fences had 2-cm spacing between the vertical conduits of the fence portion of the weir. A single collection box was used with each trap. Traps were installed in the lower portion of each tributary in early to mid-April when water temperatures were near 4°C and fished throughout the smolt migration until 5–6 days of no catch occurred. Traps spanned the entire wetted width at each location. Counting fences were operated continuously for the entire migration period in 1995, but all net traps in 1994 were inoperable for 1–3 days several times over the migratory period due to high flows (see Whalen et al. 1999*b*).

Mark–recapture

Mark–recapture was used to estimate smolt population size (Dempson and Stansbury 1991; Thedinga et al. 1994). Daily, a portion of smolts collected in the traps were double-marked with an alphanumeric visual implant tag (Blankenship and Tipping 1993) and acrylic paint applied to the postocular adipose tissue or a partial caudal fin clip. Within 24 h of tagging, marked smolts were released approximately 75–100 m upstream of each trap, typically in the late afternoon or early evening of the day of capture. Releases of tagged smolts were stratified through the migration season, with the intent of estimating trap efficiency over a wide range of conditions (Dempson and Stansbury 1991; Bagliniére et al. 1993). On four occasions during the migration period in 1995, approximately 20–40 smolts were held in flow-through cages for 48 h to assess handling mortality. No adjustment to the smolt population estimates was required because no mortality was observed in the holding tests and double-marking ensured 100% mark retention/recognition.

Table 1. Definitions of the Atlantic salmon population recruitment model parameters identified in Fig. 1 for the main recruitment and maturation pathways.

Parameter	Definition
Main recruitment	
*SS*2	Survival of age-1 parr to age-2 smolts
*SP*2	Survival of age-1 parr to age-2 parr
*SS*3	Survival of age-2 parr to age-3 smolts
Maturation	
Sm	Survival of mature age-1 parr to age 2
Si	Survival of immature age-1 parr to age 2
Pm	Probability of age-1 parr maturing
1 – *Pm*	Probability of age-1 parr remaining immature
PSm	Probability of mature age-1 parr smolting at age 2
1 – *PSm*	Probability of mature age-1 parr remaining as age-2 parr
PSi	Probability of immature age-1 parr smolting at age 2
1 – *PSi*	Probability of immature age-1 parr remaining as age-2 parr

Traps were checked at least once daily, typically during the hours of darkness between 23:00 and 05:00. All smolts were counted and marked individuals were enumerated and released downstream of the trap. A scale sample was collected from randomly selected newly captured smolts to determine age (Whalen et al. 1999*b*). All smolts were anesthetized with buffered MS 222 (100 mg·L^{-1}) when sampled for scales and when marks or tags were applied.

Derivation of smolt population estimates and densities

To derive smolt population estimates from the mark–recapture data, we used a Bayesian algorithm to determine, over a range of possible population estimates, the most probable population size given R recaptures out of M marks placed in a sample catch of C (Gazey and Staley 1986; Chaput et al. 1993). This approach is useful when population size is small (Gazey and Staley 1986) and, as with our data, when recaptures are inadequate to complete a temporally stratified analysis (Dempson and Stansbury 1991; Warren and Dempson 1995).

Age-2 and age-3 smolt density was determined by partitioning the total smolt estimate by age-class (age data from Whalen et al. 1999*b*) and then dividing the estimate by the number of 100-m^2 habitat units stocked with fry 2 years preceding the smolt migration for age-2 smolts and 3 years preceding the smolt migration for age-3 smolts. Thus, smolt densities were based on the number of habitat units initially stocked with fry for each cohort. Habitat units stocked with fry in each tributary were adjusted for habitat not sampled below the trap and the West River confluence.

Deriving survival probability distribution functions (PDFs)

Our approach for defining *SS*2, *SP*2, *SS*3, and (*SS*2 + *SP*2) incorporated variance in parr and smolt density to derive PDFs for each parameter. Our justification for this approach was that traditional analytical methods using average parr and smolt densities to define point estimates of survival (Orciari et al. 1994; Cunjak and Therrien 1998) are inadequate because point estimates of survival fail to suitably reflect the uncertainty of parr and smolt density

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Table 2. Mark–recapture data used to determine survival and probability of smolting for mature and immature Atlantic salmon age-1 parr (Whalen and Parrish 1999).

	Age-1 fall parr	Age-2 smolts	Age-2 remaining parr	Smolt ratio	Parr ratio
Mature	357 (*N*2)	15 (*N*1)	19 (*N*1*)	*N*1/*N*2 = 0.042	*N*1*/*N*2 = 0.053
Immature	424 (*N*4)	59 (*N*3)	5 (*N*3*)	*N*3/*N*4 = 0.14	*N*3*/*N*4 = 0.012
% mature	45.7	20	79		
% immature	54.3				

Note: Age-1 fall parr represent the number of mature and immature age-1 parr marked during 1994 and 1995 October–November electrofishing surveys in the Rock River. Age-2 smolts are from 1995 and 1996 recaptures of migrating individuals marked the previous fall as age-1 parr as well as age-2 smolts that perished in the trap and were dissected to determine the previous state of maturity. Age-2 remaining parr are from 1995 and 1996 June and subsequent October–November electrofishing collections of age-2 parr marked the previous fall as age-1 parr. The percent fall age-1 parr mature and immature and the smolt and parr ratios are used in the procedure outlined in the Appendix to define survival and probability of smolting for mature and immature parr.

data used to generate them. The derivation of PDFs for survival, through the incorporation of variance in parr and smolt density, enabled us to account for a wide range in the precision of parr and smolt density estimates. For parr, variance in catchment-level estimates may arise due to variability in habitat quality, whereas for smolts, variance in catchment-level estimates may arise because of the difficulty of sampling in spring high flows that affects the success of mark–recapture. We feel that our approach was well suited for the present analysis because of the difficult sampling conditions that we encountered and because the small population size of smolts in our tributaries hindered our ability to estimate smolt density with high precision.

We quantified variance for parr and smolt density data by generating PDFs from mark–recapture data for smolts and for parr using the multiple estimates of cohort density from VTFW and USFS sampling of index stations in each of the three catchments. For smolts, no additional manipulation of existing data was required to generate PDFs because a PDF for smolt population size was the end-product of our analysis of mark–recapture data using the Bayesian algorithm. The functional form of the smolt PDFs represented the precision of each smolt estimate based on the success of mark–recapture.

For parr, the procedure used to generate PDFs involved (*i*) ordering the *N* parr density estimates ($N = 5$–8 per tributary) for each cohort, (*ii*) transforming the ordered data to a cumulative distribution function (CDF), and (*iii*) fitting a mathematical function to the CDF using nonlinear regression and curve fitting (JMP® software, SAS Institute Inc. 1996). Several functions often adequately described the functional form of the CDF; we selected the most parsimonious function that adequately described the CDF. Subsequently, $N = 1000$ random samples were drawn from the CDF to generate a PDF for density for each parr cohort. For both parr and smolts, we report the maximum likelihood value of each PDF as well as 95% confidence limits (CL) representing points ≥2.5 and ≤97.5%.

We derived PDFs for *SS*2, *SP*2, *SS*3, and (*SS*2 + *SP*2) by randomly sampling ($N = 1000$) the corresponding parr and smolt density PDFs. We report the maximum likelihood value of each survival PDF as well as 95% CLs representing points ≥2.5 and ≤97.5%. Graphs of *SS*2, *SP*2, and (*SS*2 + *SP*2) PDFs were generated for qualitative comparisons of recruitment among tributaries and years.

Parr maturity recruitment pathways

Age-1 to age-2 survival includes a maturation pathway involving the recruitment of mature and immature age-1 parr to age-2 smolts or age-2 parr (Fig. 1*b*). Maturation of age-2 parr occurs, but the rarity of age-2 parr and age-3 smolts in the West River tributaries precluded mark–recapture studies needed to determine key probabilities (Appendix). Eight parameters must be determined to characterize recruitment associated with parr maturity (Fig. 1*b*). Whalen and Parrish (1999) determined age-1 parr *P*m and thus 1 – *P*m in the Rock River, Wardsboro Branch, and Utley Brook. We used mark–recapture studies, described in Whalen and Parrish (1999), completed in conjunction with the present study to determine the relative contribution of age-1 mature and immature parr to age-2 smolts and parr in the Rock River. As we outline in the Appendix and Table 2, mark–recapture data were used with estimates of *SS*2 and *SP*2 to derive *S*m, *S*i, *PS*m, *PS*i, and thus 1 – *PS*m and 1 – *PS*i.

There is no nonlethal method of distinguishing male and female parr that are immature. We compared smolt recruitment and parr survival among maturity groups (Fig. 1*b*); mature parr are all male and immature parr are mostly female.

Our initial analyses indicated no systematic differences among tributaries in the proportion of parr becoming smolts, and maturation percentages of age-1 parr in Wardsboro Branch and Utley Brook are within the range observed in the Rock River (Whalen and Parrish 1999). We therefore used mark–recapture data from the Rock River to derive recruitment parameters for the parr maturity pathway for Wardsboro Branch and Utley Brook, which presumed that parr to smolt recruitment of age-1 mature and immature parr is similar among tributaries. Using the average value for *S*m, *S*i, *PS*m, *PS*i, and *SS*3, determined from the three tributaries over the two years ($N = 6$), we calculated the proportional loss of smolts relative to no parr maturing for parr maturation percentages of 28 and 50%, the low and high estimates for the three tributaries (Whalen and Parrish 1999). An age-1 parr maturation percentage of 50% is equivalent to all males maturing assuming an even sex ratio.

Simulating effects on smolt recruitment variability

We designed a model simulation as a sensitivity analysis to determine the influence of random variation in *P*m, *S*m, *S*i, *PS*m, and *PS*i on smolt recruitment variability. From Fig. 1, we summarized total smolt recruitment for a cohort as the sum of age-2 smolts (year *i*) and age-3 smolts (year $i + 1$) as age-1 parr × ((*P*m × *S*m × *PS*m) + ((1 – *P*m) × *S*i × *PS*i)) + ((age-1 parr × (*P*m × *S*m × (1 – *PS*m)) + ((1 – *P*m) × *S*i × (1 – *PS*i))) × *SS*3), with variables as defined above. We created PDFs from the six estimates of *S*m, *S*i, *PS*m, and *PS*i using the procedure described above for the derivation of PDFs for parr density. We used 12 estimates of age-1 parr maturity determined in the three tributaries to form a PDF for *P*m (Whalen and Parrish 1999). We ran $N = 1000$ iterations of the model for each parameter, where the PDF of the parameter in question was randomly sampled, while each of the remaining four parameters were held constant, using the average value for each parameter as the constant. For each of the five parameters, we determined the coefficient of variation (CV) for the number of smolts produced for a cohort beginning with 10^5 age-1 parr: CV = SD/μ × 100 where SD is the standard deviation and μ is the sample mean.

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Data analysis

We used regression analysis to determine if the daily catch of marked smolts (dependent variable) was related to the daily catch of unmarked smolts (independent variable) to assess whether recaptures were representative of capture patterns of newly collected smolts. Regression analysis was also used to determine the significance of relationships between age-1 parr density in year i and density in year $i + 1$ of age-2 smolts, age-2 parr, and the sum of age-2 smolts and parr and to determine if the y-intercept of these relationships differed from zero (Ricker 1975). For age-1 parr to age-2 smolt recruitment, a partial F test was used to determine if sufficient evidence existed to include a quadratic term in the model. We made qualitative inferences concerning cross-tributary differences in *SS*2, *SP*2, and (*SS*2 + *SP*2) by comparing PDFs and 95% CLs. We compared *SS*2 versus *SP*2, *SS*2 versus *SS*3, (*SS*2 + *SP*2) versus *SS*3, *Sm* versus *Si*, and *PSm* versus *PSi* using a Wilcoxon rank-sum test because standard transformations failed to normalize the data. Analyses were completed using JMP® software (SAS Institute Inc. 1996) and statistical significance was considered at $\alpha = 0.05$.

Results

Over the two study years, 3282 smolts were sampled, 2654 were marked for efficiency tests, and of those, 863 (33%) were recaptured (Table 3). Trap efficiency estimates from each location and year ranged between 10 and 46% (Table 3). Excluding the Rock River in 1994, the daily catch of marked smolts was significantly, positively related to the daily catch of unmarked smolts (linear regression, all $p < 0.05$).

Parr and smolt density

Over all locations and years, a mean density of 5.7 age-1 parr·100 m^{-2} produced on average 0.9 age-2 smolt·100 m^{-2}, and a mean density of 0.9 age-2 parr·100 m^{-2} produced on average 0.2 age-3 smolt·100 m^{-2} (Table 4). Variation in smolt recruitment differed markedly among ages: age-2 smolt densities varied nearly fourfold, while age-3 smolt densities varied over ninefold (Table 4). Mean total smolt density exhibited threefold variation ranging between 0.5 and 1.7 smolts·100 m^{-2} with an overall mean of 1.0 smolt·100 m^{-2} (Table 4).

Recruitment relationships

Greater smolt densities were produced at greater parr densities (Table 5). The y-intercept of the linear fit to the parr to smolt recruitment data did not differ from zero (Table 5). Although the addition of a quadratic term slightly improved the parr to smolt recruitment model r^2 and the fit of the y-intercept to 0.02, there was insufficient statistical evidence to include a quadratic term in the model. A strong positive linear relationship was observed between age-1 parr density (year i) and density (year $i + 1$) of age-2 parr and age-2 smolts and parr (Table 5). Collectively, these results show no evidence for density dependence at the tributary scale for parr densities up to 13·100 m^{-2}.

Parr and smolt survival

The percentage of age-1 parr recruiting to age-2 smolts (*SS*2) ranged from 9 to 37% with a mean ± 95% CL of 18 ± 11% (Fig. 2*a*). There were no systematic differences in the proportion of age-1 parr recruiting to age-2 smolts among tributaries or years, as with the exception of Wardsboro Branch in 1995, considerable overlap in the *SS*2 PDFs occurred (Figs. 2*a* and 2*b*). There were also no systematic differences among tributaries or years in the proportion of age-1 parr remaining instream as age-2 parr (Figs. 2*b* and 2*c*). The mean percentage of age-1 parr recruiting to age-2 parr (*SP*2) was greater than that of age-1 parr to age-2 smolt recruitment in five of the six instances (Figs. 2*b* and 2*c*); however, differences in parr and smolt survival were not significant (Wilcoxon rank-sum test, $p = 0.34$). Generally, age-1 parr to age-2 smolt survival was more variable than survival for age-1 parr to age-2 parr. Age-2 parr to age-3 smolt survival (*SS*3) ranged between 2 and 39% (mean ± 95% CL = 25 ± 15%) and did not differ from age-1 parr to age-2 smolt survival (Wilcoxon rank-sum test, $p = 0.15$).

Mean age-1 to age-2 cohort survival, defined as the sum of *SS*2 and *SP*2, exhibited less than twofold variation (27 to 46%) and considerable overlap in PDFs among tributaries and years (Fig. 3). Age-1 to age-2 cohort survival (mean ± 95% CL = 37 ± 8%) was marginally significantly greater than age-2 to age-3 cohort survival (Wilcoxon rank sum test, $p = 0.08$).

Mature and immature parr survival and probability of smolting

For five of the six estimates of mature and immature parr survival, survival of mature parr was greater than that of immature parr (Table 6). However, mean survival of mature and immature parr did not differ (Wilcoxon rank-sum test, $p = 0.34$) (Table 6). On average, immature parr had over a threefold greater likelihood of transforming to smolts than parr that matured (Wilcoxon rank-sum test, $p < 0.005$) (Table 6). Thus, differences in smolt recruitment between mature and immature parr result primarily from a lower probability of smolting for mature parr rather than from differences in survival.

Using average estimates of parr maturity pathways in Table 3, we calculated that relative to no parr maturing, smolt production may be reduced by 21% when 50% of the age-1 parr mature. Age-1 parr maturation percentages in the Rock River, Wardsboro Branch, and Utley Brook range as low as 28%, which translates into a 12% reduction in smolt production relative to no parr maturing.

Of the five parameters used in our model simulation, immature parr survival had the highest ranked CV for total smolt production (CV in parentheses): *Si* (4.4%) > *PSm* (2.9%) > *Pm* (2.6%) > *Sm* (2.1%) > *PSi* (2.0%). Thus, although maturation of parr reduces smolt production, smolt recruitment variability is most sensitive to survival of immature parr, presumably because most smolts are produced through the immature parr pathway.

Discussion

Understanding the recruitment dynamics of the parr to smolt transformation is essential for effectively managing and conserving Atlantic salmon populations, but little information exists on how parr density relates to smolt density and how survival may vary among catchments supporting differing densities of parr. We hypothesized that winter habitat could be limiting and, perhaps, cause parr to smolt recruitment to be density dependent. This hypothesis was not

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Table 3. Mark–recapture summary statistics and Bayesian maximum likelihood total Atlantic salmon smolt population estimates, including 2.5 and 97.5% CLs in parentheses derived from a CDF of the total smolt estimates.

Tributary	No. marked	Total recaptured	Recapture percentage	Total captured unmarked	Total smolt population estimate
Rock					
1994	161	16	10	152	1530 (1056–2710)
1995	1295	594	46	1743	3800 (3564–4062)
Wardsboro					
1994	159	33	21	194	935 (716–1356)
1995	449	67	15	547	3655 (2993–4714)
Utley					
1994	228	39	17	298	1745 (1353–2460)
1995	362	114	32	348	1105 (961–1305)
Total	2654	863	33	3282	

Table 4. Atlantic salmon parr and smolt density estimates (no.·100 m^{-2}), with 95% CL in parentheses, by tributary and sample year used in the analysis of survival (*SS*2, *SP*2, and *SS*3; Fig 1*a*).

Tributary	(I) Age-1 parr	(II) Age-2 smolts	(III) Age-2 parr	(IV) Age-3 smolts	Total (II + III) Age-2 cohort	(II + IV) smolts
Rock						
1993	5.70 (3.60–7.99)		1.19 (0.66–1.67)			
1994	13.08 (9.53–16.47)	0.76 (0.43–1.23)	0.83 (0.42–1.25)	0.023 (0.013–0.038)	1.59 (1.06–2.23)	0.78 (0.45–1.26)
1995		1.37 (1.25–1.50)	3.20 (1.78–4.46)	0.21 (0.20–0.24)	4.57 (3.17–5.84)	1.58 (1.47–1.72)
Wardsboro						
1993	2.82 (1.80–3.83)		0.20 (0.12–0.26)			
1994	4.09 (3.09–4.77)	0.47 (0.31–0.66)	0.66 (0.45–0.87)	0.045 (0.030–0.062)	1.12 (0.87–1.42)	0.51 (0.35–0.70)
1995		1.50 (1.11–1.89)	0.37 (0.28–0.48)	0.24 (0.18–0.31)	1.87 (1.50–2.29)	1.74 (1.35–2.14)
Utley						
1993	4.25 (3.19–5.40)		0.65 (0.42–0.85)			
1994	4.37 (3.12–5.48)	0.72 (0.54–0.98)	1.00 (0.63–1.35)	0.24 (0.18–0.32)	1.72 (1.29–2.14)	0.96 (0.76–1.21)
1995		0.38 (0.33–0.44)	0.77 (0.55–1.00)	0.17 (0.15–0.20)	1.15 (0.92–1.37)	0.55 (0.49–0.62)
Mean (±95% CL)	5.72 (±3.90)	0.87 (±0.49)	0.99 (±0.68)	0.16 (±0.10)	2.00 (±1.36)	1.02 (±0.55)

Note: The bottom row is the mean of column means.

Table 5. Regression coefficients for recruitment relationships for Atlantic salmon (*a*) parr density in year *i* versus smolt density in year *i* + 1, (*b*) age-1 parr density in year *i* versus density in year *i* + 1 of age-2 parr, and (*c*) age-1 parr density in year *i* versus density in year *i* + 1 of age-2 smolts + age-2 parr (see Fig. 1*a*).

	Coefficient (95% CL range)	*p*	r^2	*N*
(*a*) Smolts				
Linear	β_0 = 0.17 (–0.11 to 0.45)	0.22	0.61	12
	β_1 = 0.11 (–0.12 to 0.45)	0.05		
Quadratic	β_0 = 0.02 (–0.35 to 0.39)	0.91	0.68	12
	β_1 = 0.21 (0.027 to 0.39)	0.029		
	β_2 = –0.008 (–0.022 to 0.0056)	0.22		
(*b*) Age-2 parr	β_0 = –0.39 (–1.1 to 0.28)	0.18	0.93	6
	β_1 = 0.27 (0.17 to 0.37)	0.0018		
(*c*) Age-2 smolts	β_0 = 0.09 (–0.74 to 0.92)	0.78	0.93	6
+ age-2 parr	β_1 = 0.33 (0.21 to 0.46)	0.0017		

Note: Data used to define recruitment relationships are from Table 4. The parr to smolt relation in part *a* includes age-1 parr to age-2 smolt sand age-2 parr to age-3 smolts; hence, $N = 12$. A quadratic equation, $y = \beta_0 + x\beta_1 + x^2\beta_2$, is also presented for parr to smolt recruitment for comparison.

supported, as all recruitment relationships, including parr to smolt, were best described with linear functions, and thus, no evidence was found for density-dependent recruitment. Our finding of density-independent parr to smolt recruitment likely applies to other Connecticut River tributaries because parr densities in most other tributaries are within the range

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Fig. 2. PDFs for Atlantic salmon (*a*) age-1 parr to age-2 smolt survival (*SS*2) and (*b*) age-1 parr to age-2 parr survival (*SP*2). Abbreviations indicate site (R, Rock River; W, Wardsboro Branch; U, Utley Brook) and year (94, 1994; 95, 1995). The PDFs shown for *SS*2 and *SP*2 were derived from random sampling (N = 1000) CDFs created from smolt and parr density data, as described in text. (*c*) Mean value for each PDF in Figs. 2*a* and 2*b* indicated with an inverted triangle (solid, *SS*2; open, *SP*2) with 95% CLs indicated by the extent of the solid line for each tributary and year.

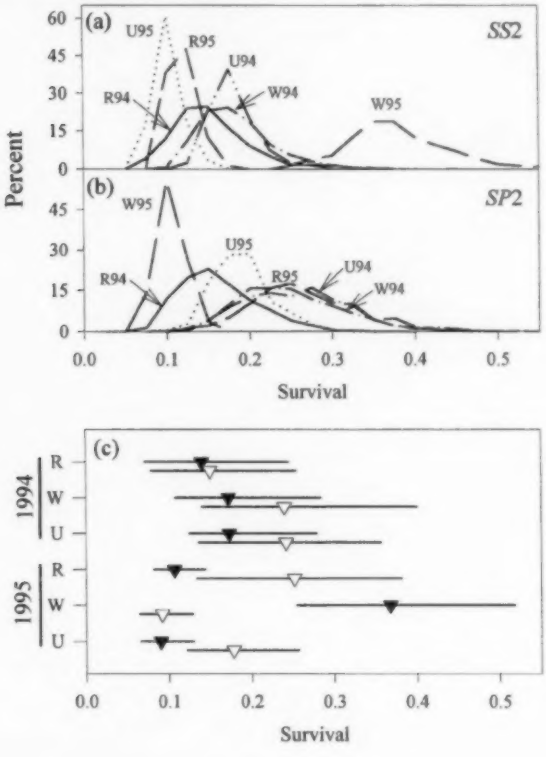
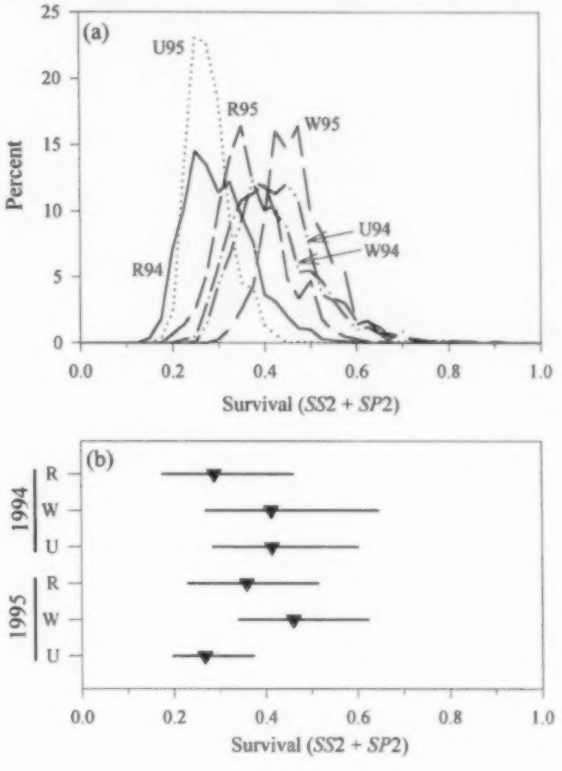


Fig. 3. (*a*) PDFs for Atlantic salmon age-1 to age-2 cohort survival (*SS*2 + *SP*2). Abbreviations indicate site (R, Rock River; W, Wardsboro Branch; U, Utley Brook) and year (94, 1994; 95, 1995). The PDFs shown for (*SS*2 + *SP*2) were derived from random sampling (N = 1000) CDFs created from smolt and parr density data, as described in the text. (*b*) Mean value for each PDF in Fig. 3*a* indicated with an inverted triangle with 95% CLs indicated by the extent of the solid line for each tributary and year.



that we evaluated (Campbell 1999). The significance of this finding for management is that for many tributaries, a simplified model where increases in parr density result in proportional increases in smolt density is sufficient to estimate smolt production from parr density data (Ricker 1975).

Studies of smolt production from fry stocking in the southern Connecticut River basin (Orciari et al. 1994) show that increased parr density, and thus greater smolt production, is achievable by stocking 100–150 fry·100 m^{-2}, two- to four-fold greater than 25–50 fry·100 m^{-2} stocking densities typically used in the West River (McMenemy 1995). Research in Vermont tributaries has indicated, however, that little incremental gain in parr production occurs when fry are stocked at >50·100 m^{-2} (Whalen and LaBar 1994; McMenemy 1995). Stocking fry at higher densities (>100·100 m^{-2}) may enhance per unit smolt production (see Orciari et al. 1994), but overall cohort survival is reduced and theoretically so is total smolt production when considered over all habitat stocked with fry (McMenemy 1995). Also, negative effects on growth that may occur at higher parr densities (Campbell 1999) could cause parr to spend additional time in freshwater to achieve a sufficient size to smolt, which is counterproductive because of the high mortality (75%) that parr experience in their third year instream. Increasing fry stocking densities to enhance smolt production must therefore be carefully evaluated, with special consideration given to the efficacy and trade-offs of increasing fry stocking density to enhance local smolt density versus maximizing overall basin-wide smolt production.

Although density dependence was not evident as a factor in the recruitment of parr and smolts at the tributary scale, we are cautious, however, not to categorically dismiss density dependence. For one, most parr densities in our study were <6·100 m^{-2}; thus, we had limited data to resolve smolt recruitment at high parr densities where density-dependent effects would have been more likely. Second, parr density in some reaches within tributaries may be as high as 20 parr·100 m^{-2}, nearly twofold greater than the highest average parr density in our analysis (e.g., Rock River in 1995 = 13 age-1 parr·100 m^{-2}). We attempted to evaluate localized density-dependent effects on smolt recruitment by

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Table 6. Probability of survival and smolting from age-1 to age-2 for mature and immature Atlantic salmon parr by tributary and smolt year.

	Survival		Smolting	
Tributary	Mature (*Sm*)	Immature (*Si*)	Mature (*PSm*)	Immature (*PSi*)
Rock				
1994	0.30	0.26	0.20	0.79
1995	0.45	0.25	0.10	0.64
Wardsboro				
1994	0.46	0.34	0.16	0.75
1995	0.31	0.58	0.52	0.94
Utley				
1994	0.47	0.34	0.16	0.75
1995	0.33	0.20	0.12	0.67
Mean (±95% CL)	0.39 (±0.09)	0.33 (±0.14)	0.21 (±0.16)	0.76 (±0.11)

Note: The bottom row is the mean of column means. Parameters are defined in Fig. 1*b* and Table 1.

individually marking parr in multiple reaches of the Rock River in October–November 1995 for recapture as smolts in 1996. However, frequent and excessive flooding in April–May 1996 negated our recapture efforts, so the issue of within-tributary parr density related effects on smolt recruitment remains unresolved. We suspect that it is possible for parr density related effects on smolt recruitment to occur at the local, reach scale without being evident at the tributary scale.

Because most smolt recruitment studies for Atlantic salmon have been completed in one tributary over time (Buck and Hay 1984; Cunjak and Therrien 1998), the spatial scale of inference of smolt recruitment data may be limited (Kennedy and Crozier 1993). Accurate modeling of smolt production requires knowledge of cross-tributary variation in smolt recruitment. We found threefold variation in smolt density among tributaries and years with no consistency for any tributary in the production of greater densities of smolts. Further, we observed consistency among tributaries in recruitment parameters; specifically, age-1 to age-2 survival exhibited less than twofold variation among tributaries and years. From our sample of West River tributaries, we conclude that any differences in smolt production arising among tributaries result primarily from differences in the parr densities that they support the preceding year. The absence of tributary effects on the proportion of parr smolting suggests that the pool of recruitment parameters that we defined may be generalized to other representative tributaries in the north-central Connecticut River basin, and perhaps to tributaries in other New England, U.S.A., river basins where Atlantic salmon fry are stocked to produce wild-reared smolts.

Other studies evaluating smolt and cohort recruitment of Atlantic salmon parr, including those on naturally spawned fish, found, similar to the results of our cross-tributary analysis, parr to smolt recruitment to range from 10 to 30% and annual survival to range from 25 to 50% (e.g., Evans et al. 1984; Cunjak and Therrien 1998). The similarity in parr to smolt recruitment and parr survival identified among Atlantic salmon populations in Canada (Newfoundland: Evans et al. 1984; New Brunswick: Cunjak and Therrien 1998) and Europe (France: Bagliniére et al. 1993) and those supported by fry stocking in the United States (Connecticut: Orciari et al. 1994; Vermont: present study) may be further indirect evidence for density independence as the primary force shaping recruitment of parr-stage Atlantic salmon. Perhaps a comparative analysis using original data from these locations would provide further insight into the forces affecting parr to smolt recruitment of Atlantic salmon.

We found no differences in survival of mature and immature parr; rather, differences in smolt recruitment may be attributed to a one-third probability of smolting for mature parr compared with immature parr. This is in contrast with results from the Little Codroy River, Newfoundland (Myers 1984), where differences in recruitment to smolts for mature and immature parr resulted from a reduction in both survival and probability of smolting for mature parr. One important difference between West River tributaries and the Little Codroy River is, with potentially rare exception, adult salmon do not spawn in our study streams, and thus, mature parr in our systems are assumed not to experience mortality associated with spawning (Hutchings and Myers 1987). It has been hypothesized that mature parr may experience greater winter mortality than immature parr (Dalley et al. 1983) because they invest more energy in gonad production than in energy storage (Rowe et al. 1991) or deplete energy reserves during fall spawning (Hutchings and Myers 1987). Recent laboratory studies where wild-reared mature and immature parr were held at 4–5°C and starved for >140 days (November–April) have found no significant differences in survival of mature and immature parr, only a difference in probability of smolting similar to that observed in the present study (K.G. Whalen and S.D. McCormick, Conte Anadromous Fish Research Center, 1 Migratory Way, Turners Falls, MA 01376, U.S.A., unpublished data). Because our results provide no support for the hypothesis that mature parr perish at a higher rate overwinter than immature parr, perhaps the disparity between our results and those of Myers (1984) indicates that the susceptibility of mature parr to winter mortality may differ among regions and thus needs to be empirically determined on a case-by-case basis for accurate modeling.

Relative to no parr maturing, our simulation showed that 21% fewer smolts may be produced when 50% of the age-1 parr are mature, which corresponds to the highest age-1 parr maturation percentages observed in the study tributaries (Whalen and Parrish 1999). The primary effect of maturation

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is to delay age at smolting; more parr that mature spend an additional year instream and therefore experience greater cumulative mortality before smolting. Although parr maturation has a significant negative effect on smolt production, sensitivity analysis on smolt recruitment variability found that the CV for smolt production for immature parr survival (*Si*) was twice as great as that for parr maturity (*Pm*). Because most smolts are produced through the immature parr pathway, changes in immature parr survival have greater effects on smolt recruitment variability than varying parr maturation percentages. This is significant because any management action designed to minimize the incidence of mature parr, such as increasing fry stocking density to minimize growth rate (see Pepper et al. 1985), will be counterproductive if it has a parallel effect on growth and (or) survival of nonmaturing parr.

Stock–recruitment analysis, which relates population density (or abundance) among life stages, is likely not sensitive to life history features such as parr maturation that strongly influence smolt recruitment but may be unrelated to parr density (but see Prévost et al. 1992). Parr maturation could therefore confound density-based stock–recruitment relationships for parr to smolt, for example, by masking density-dependent recruitment. In spite of its potential importance to Atlantic salmon population demographics (Myers 1984), the role of parr maturation has not been emphasized in models of smolt recruitment (e.g., Power and Power 1994). In concordance with the recommendations of Myers (1984), we found that quantifying smolt recruitment pathways involving parr maturation helped elucidate the population-level effect of parr maturation on smolt production and recruitment variability. We therefore strongly suggest that future model development involving Atlantic salmon parr to smolt recruitment explicitly treat parr maturation pathways.

Our approach to the derivation of survival probabilities embodied the elements of decision theory where focus is placed on the distribution of possible alternatives having varying probability rather than on sample means (Hilborn 1997). Although the biases of missed sampling were unknown, we attempted to include the uncertainty associated with the smolt and parr density estimates upon which *SS*2, *SP*2, and *SS*3 were based by randomly sampling PDFs created directly from parr and smolt density data. Thus, uncertainty was included in qualitative comparisons of *SS*2, *SP*2, *SS*3, and (*SS*2 + *SP*2) among tributaries and years (Hilborn 1997). We believe that our approach was well suited to smolt recruitment analysis that is inherently uncertain because of the difficulties of sampling. For parr, the derivation of density estimates by randomly sampling PDFs formed directly from ordered parr density data may be applicable elsewhere where similar "index station" data are available. Our approach required no a priori assumption regarding the distribution of error around parr density, which, in contrast with selecting and fixing some standard error distribution, may provide more realism in modeling applications.

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Appendix. Determination of mature and immature parr recruitment probabilities

Considering first the smolt pathway in Fig. 1, the survival of age-1 parr to age-2 smolt ($SS2$) can be expressed as

$$SS2 = (Pm \times Sm \times PSm) + ((1 - Pm) \times Si \times PSi).$$

To solve for Sm and PSm, a variable is created: let

$$\rho = (Sm \times PSm)/(Si \times PSi)$$

and thus:

$$(Sm \times PSm) = \rho \times Si \times PSi.$$

Replacing $(Sm \times PSm)$ above:

$$SS2 = (Pm \times \rho \times Si \times PSi) + ((1 - Pm) \times Si \times PSi)$$

and solving for $(Si \times PSi)$:

$$(Si \times PSi) = SS2/((\rho \times Pm) + (1 - Pm)).$$

For the smolt pathway, we define ρ as $((N1/N2)/(N3/N4))$, where $N1$ = recaptures of age-2 smolts previously mature as age-1 parr, $N2$ = mature age-1 parr marked, $N3$ = recaptures of age-2 smolts previously immature as age-1 parr, and $N4$ = immature age-1 parr marked (Table 2). Because $SS2$, Pm, and ρ are known, solve for $(Si \times PSi)$ and thus $(Sm \times PSm)$.

For the parr pathway (Fig. 1), beginning with the survival of age-1 parr to age-2 parr ($SP2$) written as

$$SP2 = (Pm \times Sm \times (1 - PSm)) + ((1 - Pm) \times Si \times (1 - PSi))$$

and procedures identical to those used for the smolt pathway, $(Sm \times (1 - PSm))$ and $(Si \times (1 - PSi))$ can be determined. For the parr pathway, variable ρ is configured slightly differently for computational ease: $((N3^*/N4)/(N1^*/N2))$, where $N3^*$ = recaptures of age-2 parr that had not previously matured at age-1, $N1^*$ = recaptures of age-2 parr that had been previously mature at age-1, and $N2$ and $N4$ are as defined in Table 2.

Using age-1 mature parr recruitment as an example, when $(Sm \times PSm)$ and $(Sm \times (1 - PSm))$ are known, PSm and thus Sm can be determined by creating two variables: let

$$V1 = (Sm \times PSm)$$

$$V2 = (Sm \times (1 - PSm))$$

and solve each for Sm:

$$Sm = V1/PSm$$

$$Sm = V2/(1 - PSm).$$

Setting equations equal based on Sm:

$$V1/PSm = V2/(1 - PSm)$$

and solving for PSm with known variables $V1$ and $V2$:

$$PSm = 1/((V2/V1) + 1).$$

Because

$$V1 = (Sm \times PSm)$$

then Sm can be defined using known terms:

$$Sm = V1/PSm.$$

Using an identical procedure, PSi and thus Si can be determined for immature age-1 parr recruitment.

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Predicted impact of zebra mussel (*Dreissena polymorpha*) invasion on water clarity in Lake Mendota

Tara Reed-Andersen, Stephen R. Carpenter, Dianna K. Padilla, and Richard C. Lathrop

Abstract: Lake Mendota, which is plagued by periodic cyanobacterial blooms, is typical of many lakes in the Laurentian Great Lakes region that are vulnerable to zebra mussels (*Dreissena polymorpha*) but have yet to be invaded. We coupled removal estimates with chlorophyll production estimates in a dynamic model to predict the likely impact of mussel-mediated removal of phytoplankton on water clarity across a range of hypothetical zebra mussel densities. Models were fit to chlorophyll and temperature data collected biweekly from Lake Mendota during 1977–1993. When we assumed daily epilimnetic circulation, the percentage of days when the chlorophyll concentration exceeded 50 $\mu g \cdot L^{-1}$ was decreased threefold at mussel densities as low as 1000 mussels$\cdot m^{-2}$. When we assumed less frequent epilimnetic circulation, the density of mussels required to substantially improve water clarity increased dramatically. We predict that zebra mussel invasion would lead to increased water clarity in Lake Mendota. Cyanobacterial blooms would be reduced but not eliminated. Negative impacts on other lake processes following zebra mussel invasion could outweigh the benefits of lower phytoplankton concentrations.

Résumé : Le lac Mendota, affecté par le fléau des efflorescences périodiques de cyanobactéries, est caractéristique de nombreux lacs de la région des Grands Lacs laurentiens qui sont vulnérables aux moules zébrées (*Dreissena polymorpha*) mais qui n'ont pas encore été envahis par cette espèce. Nous avons jumelé des estimations de l'absorption des cyanobactéries aux estimations de la production de chlorophylle dans un modèle dynamique pour prévoir l'impact probable de l'absorption du phytoplancton par les moules sur la transparence de l'eau à diverses densités hypothétiques de moules zébrées. Les modèles ont été ajustés aux données sur la chlorophylle et la température recueillies toutes les deux semaines dans le lac Mendota, de 1977 à 1993. Lorsque nous avons supposé une circulation épilimnétique quotidienne, le pourcentage de jours où la concentration de chlorophylle dépassait 50 $\mu g \cdot L^{-1}$ a été trois fois moindre à des densités de moules atteignant à peine 1000 moules$\cdot m^{-2}$. Lorsque nous avons supposé une circulation épilimnétique moins fréquente, la densité de moules requises pour augmenter sensiblement la transparence de l'eau a augmenté de façon spectaculaire. Nous sommes d'avis qu'une invasion de moules zébrées augmenterait la transparence de l'eau du lac Mendota. Les efflorescences de cyanobactéries seraient réduites, mais pas éliminées. Des effets négatifs pour d'autres processus lacustres suite à une invasion de moules zébrées pourraient l'emporter sur les avantages d'une réduction des concentrations phytoplanctoniques.

[Traduit par la Rédaction]

Introduction

Since their introduction into North America in the late 1980s (Hebert et al. 1989), zebra mussels (*Dreissena polymorpha*) have spread throughout the Great Lakes region. Factors controlling the ability of zebra mussels to invade lakes include limnological properties such as calcium concentration and pH (Ramcharan et al. 1992) and the presence of vectors for transport, most commonly recreational boat trailers (Padilla et al. 1996*a*). While zebra mussels have spread throughout the region, a large number of lakes have yet to be invaded. Lake Mendota serves as an icon for eutrophic lakes not yet invaded. An extensive time series of phytoplankton data make this lake ideal for forecasting the impacts of zebra mussel invasion of eutrophic lakes in the Laurentian Great Lakes region.

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T. Reed-Andersen[1] and S.R. Carpenter. Center for Limnology, University of Wisconsin-Madison, 680 North Park Street, Madison, WI 53706, U.S.A.
D.K. Padilla. Department of Ecology and Evolution, Life Sciences Building, State University of New York at Stony Brook, Stony Brook, NY 11794-5245, U.S.A.
R.C. Lathrop. Center for Limnology, University of Wisconsin-Madison, 680 North Park Street, Madison, WI 53706, U.S.A., and Wisconsin Department of Natural Resources, Madison WI 53703, U.S.A.

[1]Author to whom all correspondence should be sent at the following address: Department of Natural and Applied Sciences, University of Wisconsin-Green Bay, Green Bay, WI 54311-7001, U.S.A. e-mail: reedandt@uwgb.edu

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Zebra mussel invasion can cause dramatic changes throughout the ecosystem (Karatayev et al. 1997). For example, zebra mussel populations have resulted in declines in phytoplankton biomass (Caraco et al. 1997), changes in the size structure of zooplankton populations (Pace et al. 1998), and increased growth rates in benthivorous fishes (Karatayev et al. 1997). In addition to the ecological effects, zebra mussels interfere with recreation and navigation by fouling boats, boat docks, buoys, and swimming beaches (Griffiths et al. 1991). In many lakes, chlorophyll concentrations have decreased following zebra mussel invasion (Karatayev et al. 1997). In Europe, managers have introduced zebra mussels into eutrophic ponds to improve water clarity (Reeders and Bij de Vaate 1990).

It is not universally true that lakes with large populations of zebra mussels have lower overall chlorophyll concentrations than would be expected given their phosphorus loads (Mellina et al. 1995). Chlorophyll reductions coincident with zebra mussel invasion may be attributable to other factors such as reduced non-point-source phosphorus emissions (Nicholls 1997). However, zebra mussel clearance rates are high (Sprung 1995) and populations tend to be dense (MacIsaac et al. 1992). Estimates of the potential for phytoplankton removal by zebra mussel populations include reductions of 36% of the primary productivity of Lake Erie (Madenjian 1995) and filtration of water volumes equivalent to Lake St. Clair twice a day (Hebert et al. 1991) or the freshwater estuary of the Hudson River every 2 days (Roditi et al. 1996).

The amount of phytoplankton actually removed from a water body by zebra mussels may be much less than maximum filtering rates would predict because removal rates are directly related to the amount of phytoplankton available (Sprung and Rose 1988). Also, because zebra mussels are stationary in the benthos, the entire phytoplankton population of a lake is not necessarily available for removal. Therefore, circulation patterns can control the amount of phytoplankton delivered to mussels. However, most analyses to date assume thoroughly mixed water bodies.

Lake Mendota has an extensive limnological database. For this study, we have compiled a 16-year time-series of summer temperatures and phytoplankton concentrations (Lathrop and Carpenter 1992; Wisconsin Department of Natural Resources, unpublished data accessible from the North Temperate Lakes Long-Term Ecological Research Project, Madison, Wis.). This database provides a stronger empirical basis for modeling than might otherwise be possible. In addition, Lake Mendota is typical of many lakes in the Laurentian Great Lakes region. Lake Mendota is a eutrophic lake receiving between 15 500 and 67 000 kg phosphorus·year^{-1} from the surrounding agricultural basin (Lathrop et al. 1998). Ongoing non-point-source pollution reduction efforts are focused on reducing lake phosphorus concentrations, which leads to a reduction in summer blue-green algal densities and improves water clarity (Lathrop et al. 1998). The availability of long-term chlorophyll, temperature, and wind data with which to develop our model makes Lake Mendota an ideal system in which to investigate the potential effects of zebra mussel invasion on stratified, eutrophic lakes.

Lake Mendota is extremely vulnerable to invasion by zebra mussels. The water chemistry could support a dense zebra mussel population (Koutnik and Padilla 1994), and frequent boat traffic between Lake Mendota and infected waters makes it likely that the mussels will invade in the relatively near future (Padilla et al. 1996*a*). Thus, Lake Mendota offers us an excellent opportunity to make predictions based on our current understanding of zebra mussel physiology and ecology that may later be tested against postinvasion data and may potentially be generalized to a number of similar lakes. By compiling what we know of zebra mussel physiology and ecology into a predictive model, the results of which can be tested postinvasion, we test the completeness of our current knowledge and add to our future predictive capabilities.

We coupled zebra mussel bioenergetics estimates of phytoplankton clearance rates with an empirical phytoplankton growth model to predict the impact of zebra mussels on water clarity, specifically the effects of zebra mussels on the frequency of cyanobacterial blooms. Previous models of zebra mussel impacts have assumed daily mixing of the epilimnion. Although the epilimnion of Lake Mendota is frequently subject to daily mixing, complete mixing may take several days at low wind speeds (Bryson and Ragotzkie 1955). Thus, we investigated the effects of mixing frequency on the phytoplankton removal potential of zebra mussel populations.

Methods and materials

We modeled the impact of zebra mussels on water clarity based on the quantity of phytoplankton that a mussel can remove from the water column, chlorophyll production rate, the number of mussels in the population, and the proportion of phytoplankton available to the mussels at any given time. In Lake Mendota, chlorophyll concentration and water clarity are related. As chlorophyll concentration decreases, light penetration increases (Lathrop and Carpenter 1992). Lake Mendota is relatively large and deep so that, in general, water clarity in the lake is not affected by resuspension of particles from wave action near the shoreline. Therefore, chlorophyll concentration may serve as a surrogate for other particulate matter that influences water clarity. Roditi et al. (1996) have shown that zebra mussels effectively clear all particulates, including phytoplankton and seston, from the water column.

Removal

The total quantity of phytoplankton removed by a zebra mussel population includes both the total amount consumed and the amount not consumed but deposited as pseudofeces. For consumption estimates, we used the consumption component of a zebra mussel bioenergetics model by Schneider (1992). Pseudofeces production was estimated after Madenjian (1995). This method allowed us to model phytoplankton removal by individual mussels within a population as a function of weight, temperature, and seston concentration. These individual estimates could then be summed to model the phytoplankton removal by a zebra mussel population of any given density and size distribution. Zebra mussels close their shells periodically, resulting in active filtration approximately two thirds of the time (Walz 1978). Therefore, consumption and pseudofeces production estimates were multiplied by 0.67 (after Padilla et al. 1996*b*).

Consumption

The zebra mussel bioenergetics model estimates growth from

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consumption minus metabolic and reproductory costs (Schneider 1992). The consumption component of this model is

$$(1) \qquad C = a_c W^{b_c} f(T) P$$

where C represents consumption (grams chlorophyll per mussel per day, a_c is the maximum consumption rate (grams chlorophyll per gram mussel per day, W^{b_c} factors in the weight dependence of consumption (with W as the wet weight (grams) of the mussel and b_c as the dimensionless exponent of maximum consumption). The temperature dependence of respiration is included in $f(T)$, and P ranges from 0 to 1 and reflects the proportion of maximum consumption realized (Schneider 1992).

We used values for a_c (0.027 g chlorophyll·g^{-1} mussel·day^{-1}) and b_c (–0.39) from Madenjian (1995). Because zebra mussels have a linear response to increasing food concentrations up to 1250 µg chlorophyll·L^{-1} (Sprung and Rose 1988), P was modeled as (chlorophyll concentration)·(maximum removable concentration)$^{-1}$ (Madenjian 1995). The temperature-dependence function was modeled after Thornton and Lessem (1978) with parameter values taken from Schneider (1992). The function is

$$f(T) = k_a k_b$$

where

$$k_a = \frac{k_1 l_1}{1 + k_1(l_1 - 1)}$$

$$l_1 = e^{g_1(T-Q)}$$

$$g_1 = \frac{1}{T_0 - Q} \ln \frac{0.98(1 - k_1)}{0.02k_1}$$

$$k_b = \frac{k_4 l_2}{1 + k_4(l_2 - 1)}$$

$$l_2 = e^{g_2(T_1 - T)}$$

$$g_2 = \frac{1}{T_1 - T_m} \ln \frac{0.98(1 - k_4)}{0.02k_4}.$$

The temperature at each time step is given by T, T_0 (12°C) is the lowest and T_m (21°C) the highest temperature at which consumption is 98% of a_c, T_1 (32°C) is the highest temperature at which consumption is $k_4 a_c$, and Q (2°C) is the lowest temperature at which consumption is $k_1 a_c$. The empirical coefficients k_1 (0.1) and k_4 (0.02) are dimensionless. All parameter values except T are constant for this application.

To run the removal model, we relied on a database of biweekly chlorophyll samples and surface temperature measurements collected during the ice-free season on Mendota from 1977 to 1993 (Lathrop and Carpenter 1992; Wisconsin Department of Natural Resources, unpublished data, data accessible from the North Temperate Lakes Long-Term Ecological Research Project, Madison, Wis.). We set a lower bound on temperature (3°C), below which there was no removal of chlorophyll by zebra mussels. While zebra mussels survive below this temperature, filtering cilia are immobile (Reeders and Bij de Vaate 1990). For an upper thermal threshold, we chose 27°C, above which inhibition of activity has been reported (Karatayev et al. 1998). Temperatures in Lake Mendota during the ice-free season rarely exceed these boundaries. In our 16-year data set, the temperature fell below 3°C on five sampling occasions and above 27°C twice.

Pseudofeces production

By binding phytoplankton in pseudofeces, zebra mussels can remove as much or more chlorophyll than they consume (Walz 1978). We modeled pseudofeces using the proportion

$$Pf = 1.79(1 - e^{(1.346W^{-0.4}(0.247W^{0.4} - P))})$$

where pseudofeces production is consumption × Pf (grams per day) (Madenjian 1995). The values for W and P are the same as in the consumption component (see eq. 1).

Chlorophyll production

We used three measures of phytoplankton in Lake Mendota: (*i*) the amount of standing stock chlorophyll in the lake, (*ii*) a published estimate of daily summer primary production, and (*iii*) a phytoplankton growth model fit to Lake Mendota data. Most estimates of the effect of zebra mussel filtration of phytoplankton biomass have used some variant on the first (Hebert et al. 1991; Roditi et al. 1996) or second (Madenjian 1995) measure of phytoplankton abundance. By including the phytoplankton growth model in our analysis, we are able to evaluate zebra mussel impacts on phytoplankton biomass within a dynamic system.

Phytoplankton standing stock

To estimate the standing stock of chlorophyll, we multiplied its concentration by the average volume of lake water in the epilimnion. Chlorophyll concentration was estimated as the mean of samples collected biweekly during the ice-free seasons of 1977–1993 (Lathrop and Carpenter 1992; Wisconsin Department of Natural Resources, unpublished data, data accessible from the North Temperate Lakes Long-Term Ecological Research Project, Madison, Wis.). The average summer epilimnetic depth is 9 m (Soranno 1997) with a volume of 285.6 × 10^6 m^3 (Brock 1985).

Lake Mendota primary productivity

Daily summer primary production in Lake Mendota was taken from Brock (1985) who estimated that production ranged from 0.5 to 6.0 g C fixed·m^{-2}·day^{-1} during the summers of 1979–1981. We converted these production estimates into chlorophyll concentrations using an average ratio of 0.04 g of chlorophyll for every gram of carbon fixed (Reynolds 1984).

Phytoplankton growth

An empirical model for predicting chlorophyll production was fit to the 16 years of ice-free season chlorophyll data from Lake Mendota. The data consisted of chlorophyll samples taken at intervals of 14 ± 2 days and ranged from 1 to 116 µg·L^{-1} with a mean of 24.8 µg·L^{-1} and a standard deviation of 19.4 µg·L^{-1} (Lathrop and Carpenter 1992; Wisconsin Department of Natural Resources, unpublished data, data accessible from the North Temperate Lakes Long-Term Ecological Research Project, Madison, Wis.). Our goal was to find a simple empirical relationship that predicted chlorophyll concentration at each time step with minimal prediction error. To find the best model, we evaluated a family of regression models (Table 1). Because the relationship between chlorophyll sampled at time t and chlorophyll sampled 2 weeks later appeared to be roughly log-linear (Fig. 1), we constructed the models to predict log chlorophyll, denoted A (Table 1). We added a term, $\Delta t A_t$, for the effect of differences in the time between sampling. Density dependence was included as $\Delta t A_t^2$. Lake Mendota experiences a *Daphnia*-driven clearwater phase through the spring (Lathrop et al. 1999). To capture this, we added a dummy variable, D_t, valued at 1 during the clearwater phase and 0 thereafter (*Daphnia* data taken from Lathrop et al. 1999). The most appropriate day for the end of the clearwater phase was determined by fitting

$$A_{t+1} = b_0 + b_1 D_{j,t} + \varepsilon_t$$

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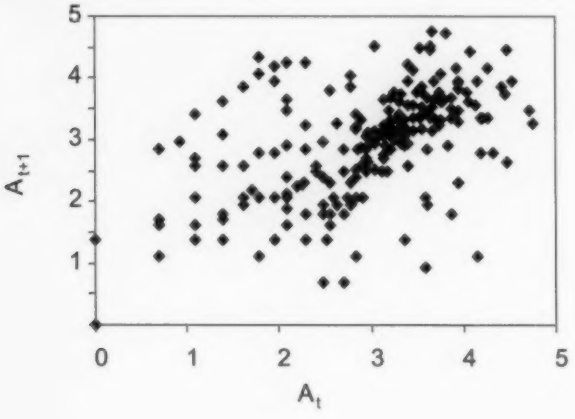
Table 1. Family of models evaluated to form the algal growth model.

Model No.	Formula	Negative log likelihood	Log likelihood ratio	AIC	r^2
1	$A_{t+1} = b_0 + \varepsilon_t$	290.96		293	0
2	$A_{t+1} = b_0 + b_1A_t + \varepsilon_t$	274.87	2:1 = **32.17**	279	0.290
3	$A_{t+1} = b_0 + b_1A_t + b_2\Delta tA_t + \varepsilon_t$	274.04	3:2 = 1.66	280	0.291
4	$A_{t+1} = b_0 + b_1A_t + b_2\Delta tA_t^2 + \varepsilon_t$	273.09	4:2 = 3.57	279	0.291
5	$A_{t+1} = b_0 + b_1A_t + b_2D_t + \varepsilon_t$	270.92	5:2 = **7.91**	277	0.334
6	$A_{t+1} = b_0 + b_1A_t + b_2\Delta tA_t + b_3\Delta tA_t^2 + \varepsilon_t$	273.26	6:3 = 1.56	281	0.291
7	$A_{t+1} = b_0 + b_1A_t + b_2\Delta tA_t + b_3D_t + \varepsilon_t$	270.1	7:3 = **7.88**	278	0.334
8	$A_{t+1} = b_0 + b_1A_t + b_2\Delta tA_t^2 + b_3D_t + \varepsilon_t$	270.11	8:4 = **5.95**	278	0.337
9	$A_{t+1} = b_0 + b_1A_t + b_2\Delta tA_t + b_3\Delta tA_t^2 + b_4D_t + \varepsilon_t$	269.02	9:6 = **8.48**	279	0.339

Note: Models are nested so that each is grown from a "parent" by an additional term. A_t is the natural log of chlorophyll concentration at time t, ΔtA_t represents algal growth between sampling times, ΔtA_t^2 represents density dependence, D_t is a dummy variable for spring grazing by zooplankton, b_i are parameters estimated by maximum likelihood, and ε_t are independent and identically distributed, $N(0, \sigma^2)$. Parameter estimation was done using maximum likelihood. A likelihood ratio of >3.84 (given in bold) implies that the additional parameter is a significant improvement (Hilborn and Mangel 1997). The model that most closely fits the data is indicated by the lowest Akaike information criterion (AIC) value.

Fig. 1. Natural log of chlorophyll concentrations at time t (A_t) versus time $t + 1$ (A_{t+1}) in Lake Mendota during the ice-free seasons of 1977–1993. Data were collected approximately biweekly.

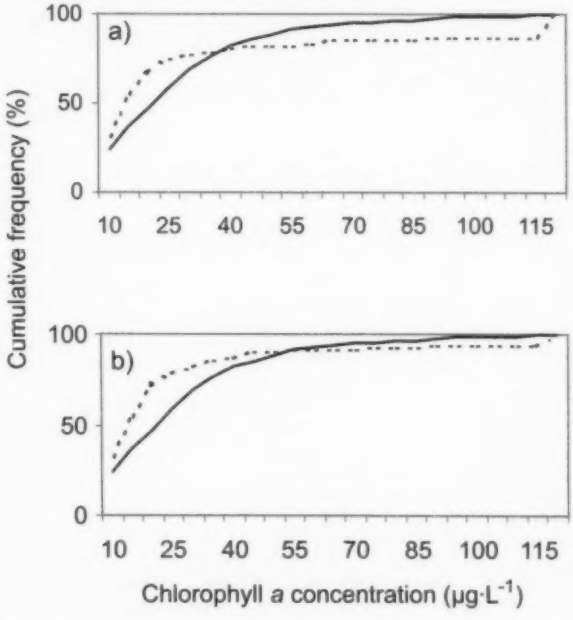


where $D_{j,t}$ is the value of D on day t when day j is the date of the end of the clearwater phase. Errors, ε_t, were normally distributed with mean 0 and homoscedastic variances. We used maximum likelihood for parameter estimation. The negative log likelihood, assuming a normal distribution, was minimized for $j = 143$.

For all models the residuals were approximately normally distributed. Model 1, that A_{t+1} was constant, was rejected on the basis of autocorrelation in the residuals. No other model showed evidence of autocorrelation as indicated by autocorrelation and partial autocorrelation function plots. Models were compared using the negative log likelihood ratio with the null hypothesis that the addition of a parameter did not significantly improve the fit of the model (Hilborn and Mangel 1997). Using this criterion, models 3, 4, and 6 were not significantly better than the next less complicated model (Table 1). As extensions of models 3, 4, and 6, models 7, 8, and 9 were also rejected. Model 5 produced the lowest Akaike information criterion value (Akaike 1973), indicating that it was the best fitting of the alternatives tested (Fig. 2). The distribution of predictions from model 5 fits observations more closely than predictions from model 2 (Kolmogorov–Smirnov statistic = 0.257 for model 5 and 0.281 for model 2).

We generated values for stochastic simulations as $\varepsilon_t = e^{\mathbf{z}_t}$ where

Fig. 2. Comparisons of the 1977–1993 data (solid lines) with simulations (dashed lines) based on two chlorophyll growth models for Lake Mendota. (*a*) Model 2 predicts chlorophyll concentrations at time $t + 1$ from a constant plus chlorophyll concentrations at time t plus a stochastic error term. (*b*) Model 5 has an additional parameter for zooplankton grazing in the spring.



$\mathbf{z}_t$ is a series of independent normal pseudorandom numbers with mean 0 and variance $\mathbf{v}_t$ calculated as

$$\mathbf{v}_t = \mathbf{x}_t' \mathbf{C} \mathbf{x}_t$$

where $\mathbf{C}$ is the covariance matrix for the parameters of model 5 and $\mathbf{x}_t$ is the predictor vector $[1, A_t, D_t]$ (Draper and Smith 1981). Technically, $\mathbf{z}_t$ should follow a Student's t distribution, but because of the large number of degrees of freedom for $\mathbf{v}$ (167), the Student's t distribution is nearly identical with the more easily simulated normal distribution.

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When we coupled the removal and production models, we used daily water temperatures interpolated from weekly samples taken in 1987, the year for which we had the most complete data.

Mussel population

The potential for zebra mussels to remove chlorophyll from Lake Mendota would depend on the number of mussels that the lake could support and the size distribution of mussels within that population (Young et al. 1996). We summarized the spatial distribution of substrates using a geographical information system (GIS) to estimate the area available for zebra mussel colonization (Fig. 3).

The size distribution of zebra mussel populations strongly influences the amount of phytoplankton that they remove. Populations dominated by smaller individuals remove substantially less phytoplankton from the water column (Young et al. 1996). We therefore identified two types of substrate in the lake likely to support different zebra mussel population size distributions. Type 1 substrates were sufficiently firm to support a mixed-age population of zebra mussels. Areas identified as type 1 consisted of rock, sand, gravel, and sandy mud (Karatayev et al. 1998). Zebra mussels also settle on macrophytes. However, because the macrophytes die back every year, the plants primarily support small mussels (Karatayev et al. 1998). We identified areas with silt bottoms and dense annual macrophyte growth as type 2 and assigned to them a zebra mussel population dominated by small (<5 mm) mussels. The total area (1100 ha) in these two substrate types was calculated using ARC/INFO (ESRI 1997).

The base coverage for the GIS was digitized from a hydrographic map of Lake Mendota (Eaton 1981). Two surveys were combined to form the sediment coverage used to estimate the area of type 1 substrate in the lake. The first, a sediment survey consisting of 50 cores taken in a 2000-m grid across Lake Mendota, was completed in 1954 (Murray 1956). We compared this with a second survey done in June 1996 to insure that the sediments had not changed substantially. For the 1996 survey, sediments were sampled along six transects around the lake at depths of 0.5, 1, 2, 4, 6, 8, 10, and 12 m. For samples taken from depths of ≤6 m, divers removed sediment from within 0.5-m^2 quadrats to a depth of approximately 10 cm. Ekman dredge samples were taken at water depths of >6 m.

A GIS coverage of macrophyte distribution was compiled from a survey completed in 1995 (Reed-Andersen 1999). Type 2 substrate was identified as the area where macrophytes were found but the substrate was not suitable for direct zebra mussel colonization. Areas where macrophytes were found with substrate that would support zebra mussels were classified as type 1 habitat.

Of the substrate available for zebra mussel colonization, 90% was composed of sand, rocks, and pebbles (type 1). The majority of this area consisted of sand and mud comparable with the substrate populated by zebra mussels in western Lake Erie reported by Berkman et al. (1998). The remaining 10% of available substrate was macrophytes (type 2). In the 1995 macrophyte survey, the most common plant found in these sites was Eurasian water milfoil (*Myriophyllum spicatum*), which was present at 64.5% of the sampling depths at which macrophytes were found (Reed-Andersen 1999). The next most common was coontail (*Ceratophyllum demersum*) at 47.3%. These tall plants could be expected to provide ample settling sites for zebra mussel veligers. The total available habitat including type 1 and 2 substrates was approximately 1 100 000 m^2, or 27.6% of the lake. Although Berkman et al. (1998) reported small zebra mussel populations on sediments with grain sizes <16 μm, Lake Mendota gyttja has much smaller particles (Brock 1985, p. 55); therefore, it is unlikely to support a mussel population (Karatayev et al. 1998). For each habitat type, mussel size distributions were modeled from mixed-age (type 1) and predominantly young (type 2) populations reported from Saginaw Bay (Nalepa et al. 1995). The predominance of type 1 habitat led to a simulated zebra mussel population of mixed age and a mean length of 13.3 mm.

Circulation

Physical modeling of water circulation in the lake is beyond the scope of this paper. A circulation model for Lake Mendota does not exist. Early studies of water movement in the lake suggest that the epilimnion may circulate completely about every 3 days (Bryson and Ragotzkie 1955). We used this as a crude estimate of the impact of imperfect mixing on the ability of zebra mussels to remove phytoplankton. We assumed that a third of the chlorophyll in the lake would come in contact with the nearshore areas and be available to zebra mussels on any given day. Because information about circulation patterns in Mendota is incomplete, we also evaluated the model at several lake circulation times ranging from 1 to 100 days.

Currents in Lake Mendota are primarily wind driven (Haines and Bryson 1961). In most summers, approximately 95% of the phytoplankton standing crop is cyanobacteria capable of buoyancy control (Lathrop and Carpenter 1992). Cyanobacteria floating on the surface of the water would be unavailable for zebra mussel removal. Soranno (1997) found that cyanobacterial blooms in Lake Mendota in the summer and fall of 1993 occurred on days when the average wind velocity for Madison was <2.68 $m \cdot s^{-1}$. We modeled this pattern by assuming that only 5% of the chlorophyll was accessible on days when the average wind speed fell below 2.68 $m \cdot s^{-1}$. The Wisconsin State Climatologist provided daily wind data collected at the Dane County Regional Airport, approximately 3 km from Lake Mendota. Average daily wind speed was calculated as the sum of daily hourly wind speeds divided by 24.

Evaluation of zebra mussel impact on water clarity

We used a three-step process to evaluate the possible impact of zebra mussels on the water clarity of Lake Mendota. We first compared daily estimates of chlorophyll removal with static assessments of the chlorophyll standing stock in Lake Mendota and the estimated primary productivity. We then combined the removal component and the phytoplankton growth component into a single dynamic model so that in each time step, chlorophyll was both removed and produced. Model runs extended from May 1 to October 31 (184 days). Average chlorophyll concentrations in Lake Mendota for this time period ranged from 9.28 to 37.25 $\mu g \cdot L^{-1}$. We began each model run with an initial chlorophyll concentration of 30 $\mu g \cdot L^{-1}$. Daily temperature values were based on interpolation of 1989 surface temperature values for Lake Mendota. Finally, we modified the dynamic model to account for intermittent circulation. At each postulated zebra mussel density, we ran the model five times for every circulation periodicity (including complete daily circulation).

For all three approaches, we ran the model assuming a range of zebra mussel densities. In North America, local zebra mussel densities can range from 100 to >10 000 $\cdot m^{-2}$ (Hebert et al. 1989; Mellina et al. 1995). For each density estimate, removal estimates were summed across both substrate types.

Results

A common method for estimating the impact of zebra mussels on a given water body has been to compare potential removal with the average phytoplankton standing stock (e.g., Hebert et al. 1991; Roditi et al. 1996). When we applied this method to Lake Mendota, we found that at densities of >2000 mussels $\cdot m^{-2}$, a zebra mussel population could remove an amount of phytoplankton equivalent to the mean lake standing stock (Fig. 4). However, removal of phyto-

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Fig. 3. Potential zebra mussel colonization sites within Lake Mendota. Firm substrate (type 1, maximum depth 8 m) could support a mixed-age population. Macrophyte substrate (type 2, maximum depth 4 m) could support a zebra mussel population dominated by small (<5 mm) mussels. Colonization of gyttja is unlikely. Maximum freezing depth rarely exceeds 0.75 m.

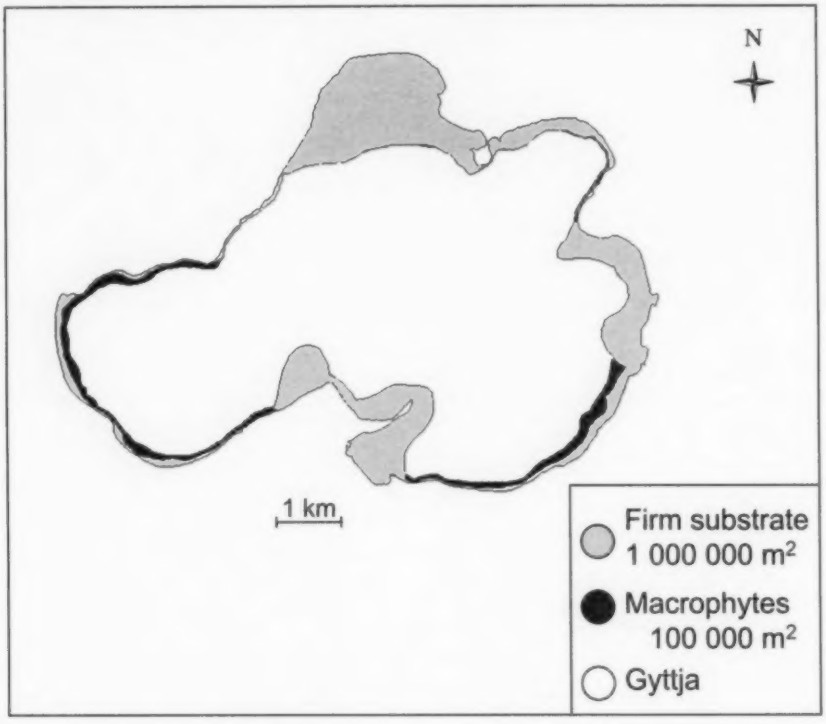
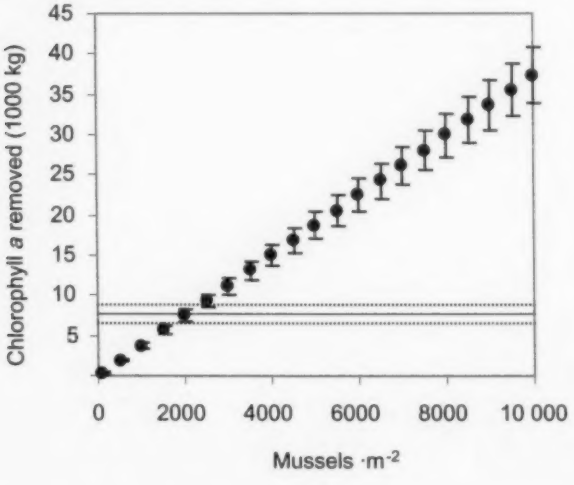


Fig. 4. Chlorophyll removal potential for zebra mussel populations across a range of densities. Points represent the modeled mean and standard error daily chlorophyll removal potential for each mussel density. The lines represent the mean and standard error of whole-lake chlorophyll in Lake Mendota during the ice-free seasons of 1977–1993.



plankton from the water column is only likely to impact water clarity when the amount removed exceeds production.

Therefore, Brock's (1985) estimates of daily production during 1979–1981 were compared with removal estimates generated from the removal component of our model and based on chlorophyll and temperature data from that same period. We found that, within a 2-week period, even at a zebra mussel density of 500 mussels·m^{-2}, removal potential often exceeded production (Fig. 5). This was particularly true at midsummer temperatures.

We developed the phytoplankton production model to allow us to estimate the relationship between daily removal and production. Model runs with mussel densities of 0 should produce similar frequencies as were found in the data. The production model predicted fewer intermediate values (25–40 μg·L^{-1}) than exist in the data (r^2 = 0.33) but fairly accurately predicted the number of days when chlorophyll concentrations exceed 50 μg·L^{-1} (Fig. 6). Potential bloom conditions exist in Lake Mendota at concentrations exceeding 50 μg·L^{-1}. Therefore, we chose this as the currency for the coupled production and removal model.

When we coupled removal and production, low densities of zebra mussels (100 mussels·m^{-2}) did not appear to impact chlorophyll concentrations (Fig. 6). However, moderate densities of zebra mussels (500–1000 mussels·m^{-2}) removed enough phytoplankton to reduce the occurrence of chlorophyll concentrations exceeding 50 μg·L^{-1}. At densities of >2000 mussels·m^{-2}, chlorophyll concentrations rose above 50 μg·L^{-1} <1% of the time.

When we assumed mixing of the epilimnion every 3 days,

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Fig. 5. Comparison of chlorophyll removal and production estimates for Lake Mendota. (*a*) Brock's (1985) production estimates for the ice-free season in 1979 (circles), 1980 (triangles), and 1981 (squares); (*b*) removal estimates modeled using temperature and chlorophyll data from the same years.

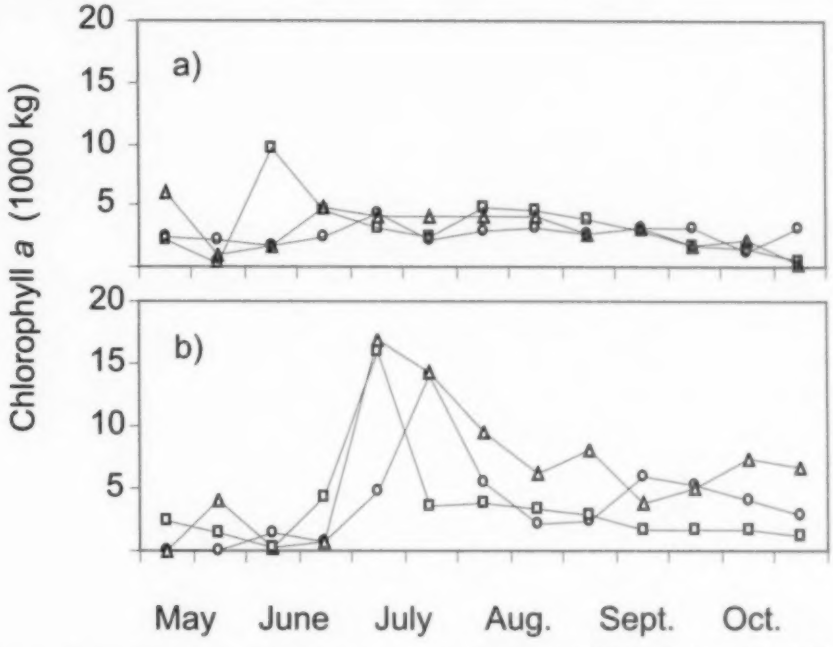
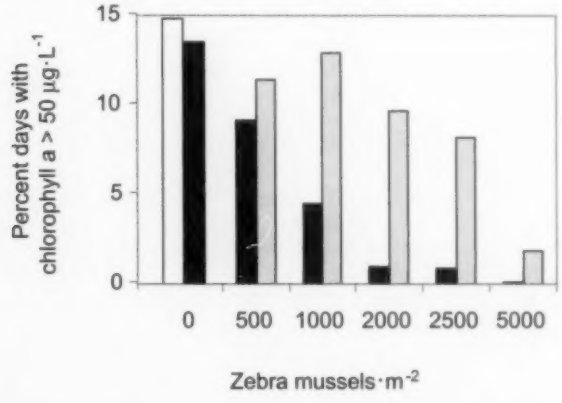


Fig. 6. Results of the coupled removal and production models summarized as the percentage of days when chlorophyll concentrations exceeded 50 $\mu g \cdot L^{-1}$, indicating a potential cyanobacterial bloom. Solid bars represent model runs assuming daily epilimnetic circulation. Hatched bars represent model runs for which we assumed that each day, 33% of the chlorophyll was available for removal by zebra mussels (circulation time 3 days). For reference, an open bar is presented that summarizes the percentage of days when Lake Mendota chlorophyll concentrations exceeded 50 $\mu g \cdot L^{-1}$ during the ice-free seasons of 1977–1993.



the mussel density sufficient to impact water clarity substantially increased (Fig. 6). The model assuming complete circulation predicted that a zebra mussel population of 2000·m^{-2} would reduce the number of days when chlorophyll concentrations exceeded 50 $\mu g \cdot L^{-1}$ to <1%. With an epilimnetic circulation period of 3 days, the mussel density required to achieve this reduction was more than twice that required when we assumed complete circulation.

At densities of <5000 mussels·m^{-2}, increasing the circulation period to once every 7 days produced results similar to those of the 3-day circulation model (Fig. 7). At higher zebra mussel densities, the percentage of days with chlorophyll concentrations above 50 $\mu g \cdot L^{-1}$ was greater with circulation every 7 days than with circulation every 3 days. At circulation periods >30 days, even mussel densities of 10 000·m^{-2} had minimal impact on the frequency of chlorophyll concentrations 50 $\mu g \cdot L^{-1}$.

Assuming daily epilimnetic circulation, temporal variability in chlorophyll concentration declined substantially with increased mussel density (Fig. 8). With no zebra mussel predation, the coefficient of variation (CV) was 1.26 ± 0.05. For the model assuming complete circulation, the mean CV for 5 simulated years decreased from 1.21 at a density of 500 mussels·m^{-2} to 0.53 at 5000 mussels·m^{-2}.

Population density also determined how increasing the circulation period affected the variability of chlorophyll concentration. For a zebra mussel population of 2000 mussels·m^{-2}, the circulation period had no impact on the variability of chlorophyll concentrations (Fig. 9*a*). However, with a dense zebra mussel population (10 000 mussels·m^{-2}), variability increased with circulation period, approaching the variability observed with no mussels (Fig. 9*b*).

Discussion

Most estimates of phytoplankton removal potential have

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Fig. 7. Percentage of potential bloom days (chlorophyll >50 $\mu g \cdot L^{-1}$) across a range of zebra mussel densities modeled given various circulation periodicities. A circulation time of 100 days indicates that 1% of the chlorophyll was available to the mussels in each time step.

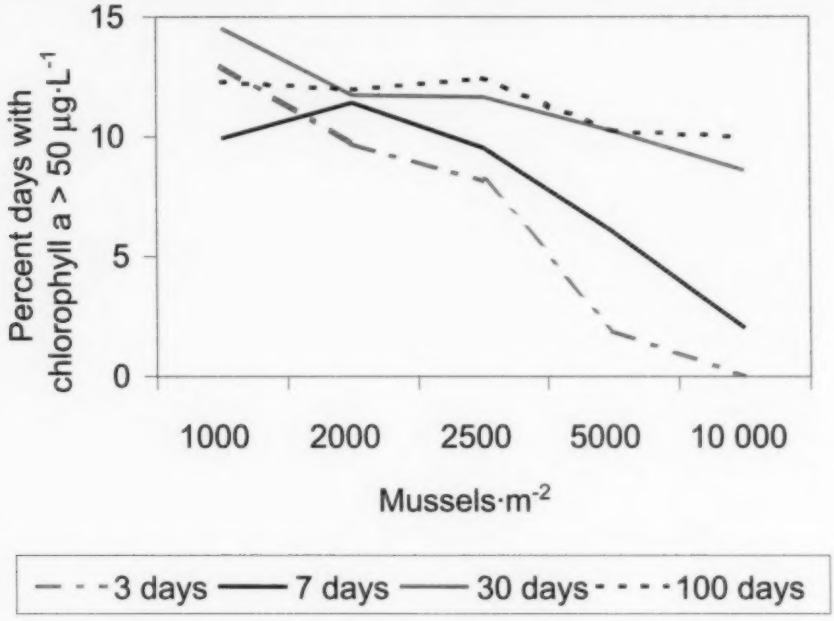
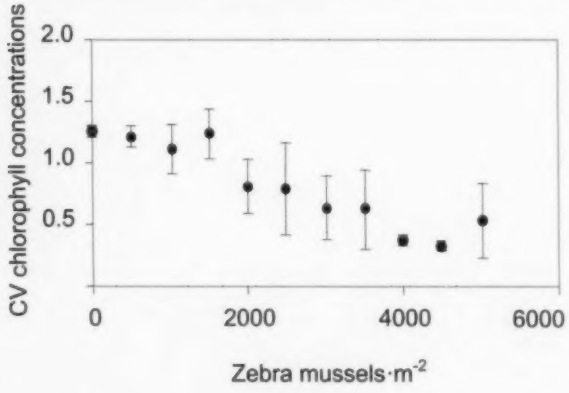


Fig. 8. CV in modeled chlorophyll concentrations at zebra mussel densities of 0–5000 mussels·m^{-2}, assuming daily circulation of the epilimnion. Data points represent mean CV; error bars are the standard deviation in five simulated years.



compared removal estimates based on field or laboratory experiments with independent estimates of phytoplankton biomass or primary productivity (Madenjian 1995). These studies indicate that mussel populations are capable of removing a substantial proportion of the chlorophyll. When we use these same techniques to predict zebra mussel impact on Lake Mendota, our results agree. However, when we incorporate daily phytoplankton production in the analysis, we find that while relatively small zebra mussel populations may increase overall water clarity, even dense zebra mussel populations do not remove the possibility of occasional chlorophyll concentrations high enough to presage cyanobacterial blooms.

By coupling the phytoplankton growth and removal estimates dynamically, we were able to evaluate the potential chlorophyll removal given changes in the chlorophyll concentration at each time step. This calculation approximates natural conditions where, if the mussels remove enough phytoplankton to impact the standing stock on one day, less is available to them the next. This feedback decreased our estimates of the total amount removed. However, because the reductions in chlorophyll concentration were carried over from one day to the next, an impact on phytoplankton production was predicted at lower zebra mussel densities than when we assumed that chlorophyll concentrations remained static. Our simulations indicate that, for a homogeneously mixed lake, substantial reductions in chlorophyll concentrations could occur at densities of around 500 mussels·m^{-2}. These findings agree with the results of a biomanipulation project involving zebra mussels (Reeders and Bij de Vaate 1990). In three eutrophic lakes, Reeders and Bij de Vaate (1990) found that the density of zebra mussels required to reduce local phytoplankton biomass ranged from 540 to 675 mussels·m^{-2}.

The removal potential of a zebra mussel population is a function of the prodigious filtering capabilities of zebra mussels. However, this is mediated by the availability of phytoplankton for removal. Therefore, actual removal also depends on wind patterns, currents, and mixing. Because of soft midlake sediments in Lake Mendota, mussels would be restricted to nearshore areas. The impact of intermittent mixing would be to reduce the amount of phytoplankton available for removal. This could create a refuge for cyanobacteria in the deep waters, especially on calm days when improved water clarity inshore could be coupled with

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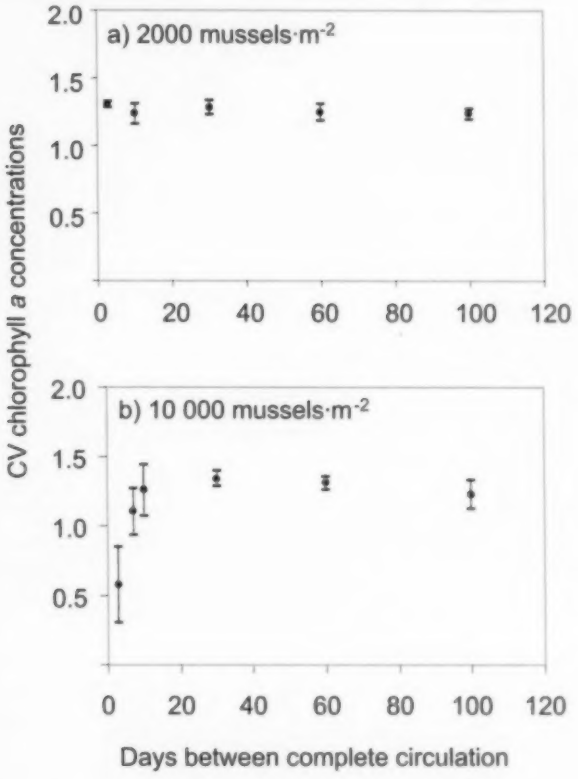
Fig. 9. CV in modeled chlorophyll concentrations across a range of circulation periods for zebra mussel densities of (*a*) 2000 and (*b*) 10 000 mussels·m^{-2}. Data points represent mean CV; error bars are the standard deviation in five simulated years.



midlake cyanobacterial blooms. *Aphanizomenon* bloom periodically in Oneida Lake (Horgan and Mills 1997) despite reduced lake-wide chlorophyll concentrations following zebra mussel invasion (Mellina et al. 1995). Once cyanobacterial blooms occur, mussel populations could be expected to have little impact on water clarity.

In the absence of bloom conditions, zebra mussels could be expected to remove substantial quantities of cyanobacteria. Reeders and Bij de Vaate (1990) found that the filtration rate of the zebra mussels was unaffected by cyanobacterial concentrations. In fact, recent studies indicate that zebra mussels may preferentially remove cyanobacteria (Lavrentyev et al. 1995; Baker et al. 1998; Bastviken et al. 1998). However, zebra mussels are unlikely to entirely remove cyanobacteria from the water column.

The periodicity of epilimnetic mixing has a strong influence on the availability of phytoplankton to mussels, and therefore on water clarity. During extended calm periods, even a dense population of mussels would be unlikely to improve water clarity. In a 3-year study of water circulation in a large bay of Lake Mendota, Bryson and Ragotzkie (1955) found that water replacement frequently required less than a day. However, they also reported replacement times of 5 and 28 days. We expect therefore that invasion of zebra mussels into Lake Mendota could lead to periods of both increased water clarity and severe midlake blooms. For lakes, studies that assume complete daily mixing of the water body may overestimate the actual potential of zebra mussel populations to remove phytoplankton. It is important to note that chlorophyll concentrations consistent with bloom conditions were not entirely absent in any simulations.

Following zebra mussel invasion, other impacts on the lake could easily outweigh any benefits to water clarity. Because buoyancy gives cyanobacteria a refuge from zebra mussel predation, these undesirable phytoplankton would be least impacted. During blooms, selective predation by mussels could lead to an even greater dominance of cyanobacteria in Lake Mendota. In addition, lower chlorophyll concentrations in the pelagic zone could have unexpected consequences for the food web.

In other systems, both available phosphorus and light penetration have increased following zebra mussel invasion (Caraco et al. 1997). Increased light and nutrient availability would lead to greater phytoplankton production and could increase the probability of cyanobacterial blooms. It is interesting to note that zebra mussels thrive in oligotrophic and mesotrophic lakes but are less abundant in extremely eutrophic systems (Karatayev et al. 1998). One of the ironies of zebra mussel invasion could be that increases in water clarity would undermine popular support for reduction of non-point-source pollution. Yet, in the long term, the ongoing management efforts aimed at reducing non-point-source phosphorus pollution are likely to provide a more sustainable solution for reducing nuisance blooms in eutrophied lakes.

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European eel (*Anguilla anguilla*): prediction of spawner escapement from continental population parameters

Eric Feunteun, Anthony Acou, Pascal Laffaille, and Antoine Legault

Abstract: This paper describes the assessment of silver European eel (*Anguilla anguilla*) escapement based on a "sedentary" population fraction analysis in a 60-km^2 watershed of northern Brittany (France). Downstream migration fluxes were monitored using eel traps and related to environmental factors. Intensive electrofishing and fyke-net fishing were conducted to assess eel biomass, densities, and population structure. A total of 564 eels, including 81 silver eels, were PIT tagged. In 1996, 616 eels were caught in the catchment including 68 silver eels (11%). During the following downstream migration period, 12 of the PIT-tagged silver eels, among a total of 678, were recaptured in the downstream traps. Seven were recaptured in the catchment in 1997. It was shown that (*i*) only about 20% of the silver eels present in the catchment emigrated during the following year, (*ii*) 12% stayed in the catchment including two (3.4%) that recovered yellow eel characteristics, and (*iii*) the other eels either died or settled in the catchment but were not recaptured. It was also estimated that 2% (650 eels) of the population (34 000 eels) among 3000 silver eels considered "emigration candidates" emigrated each year,.

Résumé : Le but de cette note est de proposer une approche permettant d'estimer l'échappement des anguilles européenne argentées (*Anguilla anguilla*) basée sur une analyse de la fraction de la population sédentaire dans un bassin versant de 60 km^2 de l'ouest de la France. Les flux de migration catadrome ont été observés en utilisant des pièges à anguilles et ont été mis en relation avec les facteurs environnementaux. Des campagnes de pêche électrique et de verveux ont permis d'estimer les biomasses, densités et structures de la population d'anguilles. Cinq cents soixante-quatre anguilles, incluant 81 anguilles argentés, ont été marquées par PIT tag. En 1996, 616 anguilles comprenant 68 argentées ont été capturées dans le bassin versant. Lors du pic de migration, parmi les 678 argentées capturées dans le piège de dévalaison, 12 anguilles argentées marquées ont été recapturées. Sept anguilles argentées ont été recapturées dans le bassin versant en 1997. Ces résultats montrent que (*i*) seulement 20% des anguilles argentées présentes dans le bassin versant migrent dans l'année, (*ii*) 12% restent dans le bassin versant, dont 2 (3.4%) qui régressent au stade jaune et (*iii*) les autres anguilles meurent où se sédentarisent dans le bassin versant. Le stock total d'anguille du bassin versant est de 34 000 anguilles, parmi celles-ci, on estime à environ 3 000 individus le stock d'anguilles argentées considérées comme « candidates à l'émigration ». Mais seules 650 anguilles argentées dévalent réellement, soit environ 2% du stock.

Introduction

Since the 1980s, the European eel (*Anguilla anguilla*) stock has declined throughout its distribution range, including all accessible continental and coastal hydrosystems that link with the Baltic Sea, the North Sea, the Channel, and the Atlantic and Mediterranean coasts between Iceland and Morocco (Moriarty and Dekker 1997). The eel is one of the major components of many estuarine and fluvial aquatic systems. It has an important commercial value throughout Europe (about Euro 180 million·year^{-1}), and its biological cycle is long (5–18 years) and remains insufficiently known to develop reliable sustainable management policies.

The European eel dominates fish communities of many inland aquatic systems. For instance, it represents more than 50% of the fish biomass in estuarine systems such as lagoons (Feunteun 1994; Feunteun and Marion 1994) and in rivers of the Mediterranean or Atlantic coast, at least in their downstream reaches (Chancerel 1994; Feunteun et al. 1998). The species is found in practically every type of adequate habitat, and it is quite often the only species that occurs in shallow waters unsuitable for any other species.

All species of eels (*Anguilla* spp.) are highly migratory amphihaline species. Since Schmidt's (1922) work, spawning in European eel is thought to take place somewhere in the Sargasso Sea. At their arrival on the continental shelf, leptocephali larvae metamorphose into glass eels, which

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E. Feunteun[1] **and P. Laffaille.** Université de Rennes I, UMR 6553 "Ecobio," Laboratoire d'Evolution des Systèmes Naturels et Modifiés, avenue du Général Leclerc, 35042 Rennes Cedex, France.
A. Acou. Université de Rennes I, UMR 6553 "Ecobio," Laboratoire d'Evolution des Systèmes Naturels et Modifiés, avenue du Général Leclerc, 35042 Rennes Cedex, France, and Fish-Pass, 91 rue de Saint-Brieuc, 35000 Rennes, France.
A. Legault. Fish-Pass, 91 rue de Saint-Brieuc, 35000 Rennes, France.

[1]Author to whom all correspondence should be addressed. e-mail: eric.feunteun@univ-rennes1.fr

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start colonising continental waters. The growth stage lasts 3–8 years in males and 5–15 years in females. After this growth stage, yellow eels lose their yellow pigments, their back becomes dark brown or black and their belly becomes white, the eye volume increases, and the pectoral fins grow longer. All these changes are characteristic of a second metamorphosis into silver eels adapted to live in deep marine waters. After a few months, silver eels start their downstream migration towards marine waters and eventually return to the spawning grounds in the Sargasso Sea. Silver eels leaving continental waters are considered to be maturing fish, gonad maturation occurring in oceanic waters (Fontaine 1994).

Therefore, little is known about what happens between maturing silver eel stages and subsequent return of glass eels to the Northeast Atlantic and Mediterranean coasts. This lack of knowledge is at the origin of a controversy about the existence of a stock–recruitment relationship (Gascuel and Fontenelle 1994). ICES (1998) considers that the species is outside safe biological limits, and therefore, application of the precautionary principal is necessary. In particular, the ICES/EIFAC working group on eels recommended the enhancement of escapement for glass eels, yellow eels, and silver eels and the fixing of standard escapement objectives at the international level (ICES 1998).

This recommendation is weakened by the fact that the status of the stock is poorly assessed by a number of national or regional surveys of fluvial recruitment and that nothing is known about the minimal number of glass eels needed to sustain sufficient eel population and subsequent silver eel emigration (Feunteun and Vigneux 1998). Even weaker is the knowledge about silver eel escapement and related fisheries. Monitoring methods already exist and have been used for glass eels in a range of systems throughout Europe (e.g., Gascuel et al. 1994; Legault 1994; Moriarty and Dekker 1997), but no such approach has been conducted for silver eels.

The aim of our paper is to propose an approach to assess, at the scale of a whole river system, relationships between silver eel migration dynamics, structure of the "sedentary fraction" of eel populations, and environmental factors.

Material and methods

Study site

The Frémur (Fig. 1) is a small river of northern Brittany (France) that opens into the British Channel next to Saint Malo. Its catchment covers about 60 km^2, and the overall length of the river and its tributaries is 45 km, comprising 17 km for the main stream. The slope varies between 0.1 and 2% with an average of 0.6%. Despite its small size, the Frémur provides for a wide range of habitats from high-velocity streams of the trout zone to lentic waters of the bream zone in downstream areas, human-made ponds and reservoirs, wetlands, etc. The total extent of water is 75 ha including 5 ha of running waters (streams) and 70 ha of still waters (ponds and reservoirs). Therefore, this river appears to be representative of small coastal catchments of western France.

A total of six dams and weirs are present on the catchment. Three of them were totally impassable until they were equipped with eel passes (Feunteun et al. 1998). The most important one, Bois Joli, was built in 1991. It is 14 m high and creates a 3×10^6 m^3 reservoir to provide the county with drinking water. Most of the time, it is not full and the flow is controlled by pipes. When it is full, the water flows over a discharge weir. It has been equipped with an eel lift to restore upstream migration, but no equipment has been provided for downstream migration (Fig. 1).

Sedentary stock assessment

Electrofishing was conducted in 30-m-long stream sections delimited by 3-mm-mesh stop nets. A "heron" (Lamarque et al. 1978) apparatus was used. It delivered direct current (150–365 V and 0.8–6 A). According to the width and the depth of river sections, fishing was conducted either wading in the water or from a boat (0.09% of the stations). A standardised depletion method (Feunteun 1994; Lambert et al. 1994; Feunteun et al. 1998) was used to assess fish abundance (number and grams per square metre) using a Carle and Strub (1978) estimator. In September 1996, a total of 34 river sections were sampled between the estuary and streams located 15 km upstream, covering 2.4% of the available river length. In September 1997, 32 stations were sampled, covering a total of 2.1% of the river length. In the deepest habitats (ponds and reservoirs), we also used unbaited fyke nets (3-mm mesh). Fyke-net results were used to describe the population structure but were not used for stock assessment. A total of five sites were sampled with fyke nets in 1996 and six in 1997, all located in the Bois Joli reservoir. In spring 1997, a limited campaign using electricity was conducted to test marking efficiency and also permitted the recapture of a few fish marked in 1996.

Eels were measured (length and weight) and released directly outside the sampled river section immediately after their capture. Silver eels were identified using three criteria: colour of the back and belly, presence of a well-marked lateral line, and eye diameter according to Pankhurst (1982). If one of the three criteria (most often colour) did not occur, the eel was identified as yellow/silver. If only one (generally eye diameter) or none occurred, the eel was noted as yellow. Considering the shallowness of the stream, catchability was very high (on average, 70% of the standing stock was caught at the first removal). Therefore, the efficiency of the method appeared to be very good for eel sampling (including large eels), as has been stressed in previous studies (e.g., Feunteun 1994; Lambert et al. 1994; Feunteun et al. 1998).

PIT tags

All eels >200 mm, including silver eels, were marked individually using PIT tags (Prentice et al. 1990). The PIT tags were injected with a syringe into the general cavity. Induced mortality and PIT tag rejection were tested according to Prentice et al. (1990), Haines and Modde (1996), and Baisez (1997). Fourteen percent of the tags were rejected within an hour after the injection (Parker 1995; Baisez 1997). After this period, tag losses were very low. Therefore, the eels were kept for at least an hour after tagging before they were released in the river.

Downstream migration dynamics

A trap was installed on the Pont es Omnès dam; it was designed to capture every descending eel >200 mm under practically every flow condition. Daily monitoring was conducted between September 1996 and June 1998 in order to count the number of descending eels. Each fish was measured, weighed, and the presence of PIT tags detected using a magnetic detector. Environmental parameters (temperature, flow, rainfall, etc.) were measured.

A total of 19 escapement tests were regularly conducted by releasing eel batches (19–57 fish) at the top of the downstream trap of Pont es Omnès. The escapements were about 0% except during a very short period (5 days) when the river flow exceeded 1500 $L \cdot s^{-1}$. The water overflowed the collecting gutter, permitting eels to escape. Then the average escapement amounted to 37% (9–67%). The daily eel catches were corrected for the daily escapement rates.

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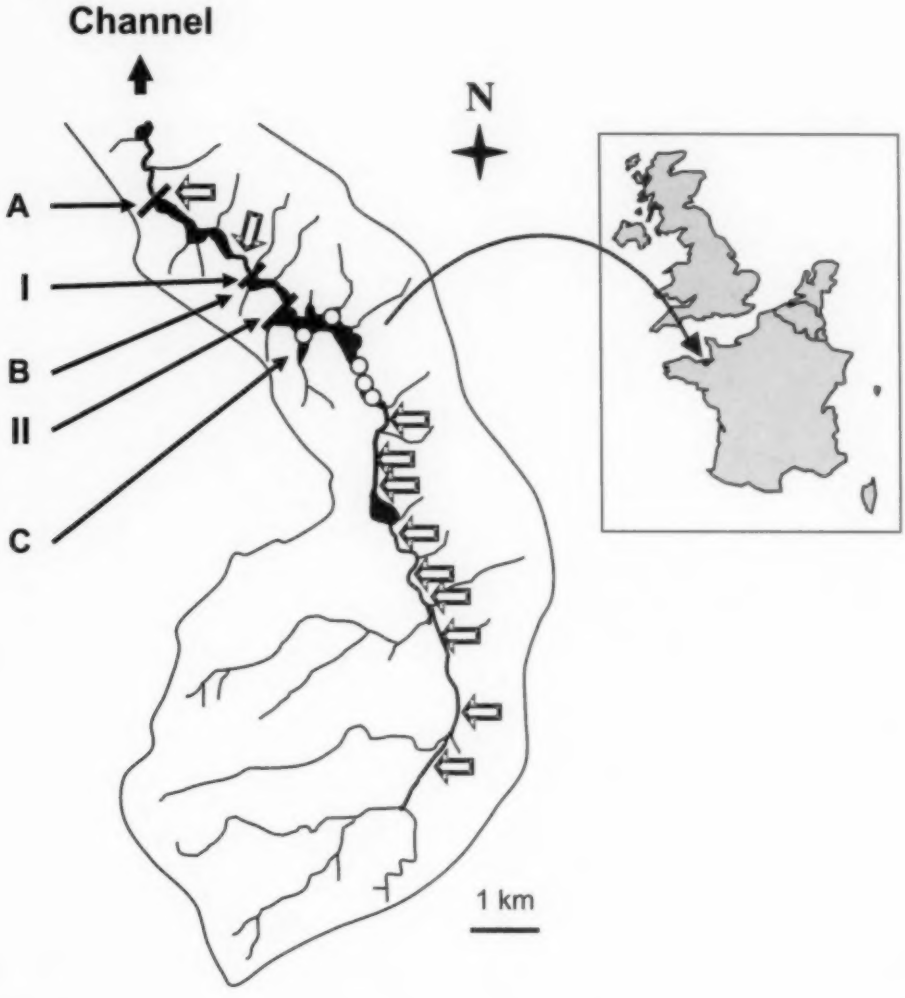
Fig. 1. Location and characteristics of the Frémur's catchment. Open arrows represent batches of two or three river sections sampled by electrofishing. Circls represent the locations of fyke-net fishing. Bars indicate the locations of major dams. A, Pont Avet reservoir (2 km from the river mouth); B, Pont es Omnès reservoir (4.5 km from the sea); C, Bois Joli reservoir (6 km from the sea); I, silver eel trap; II, Bois Joli dam.



Population size estimates

Population size was estimated by two means: extrapolation from Carle and Strub (1978) estimates and the mark–recapture method. The average density and biomass estimated in sampled river sections by electrofishing were extrapolated to the whole stream surface using the Carle and Strub (1978) estimation. The size of the silver eel stock in the basin was calculated from the size of the total silver eel run (trap data) and the fraction of the tagged population recaptured in the run (recapture data) using

$$S = N_{se} \times n_{se}/m$$

where S is the size of the silver eel stock, N_{se} is the number of silver eels tagged, n_{se} is the number of silver eels caught in the trap, and m is the number of marked silver eels recaptured in the trap.

Results

Sedentary fraction of the population

The population comprised eels between 60 and 780 mm (Fig. 2). In both years, a low proportion of elvers (young recently pigmented eels) was observed, confirming the low recruitment of glass eels due to physical obstruction of the river by major dams (Feunteun et al. 1998). In 1996, the strength of the 300- to 420-mm size-class seemed to be higher than in 1997 with, respectively, 40% and only 25% of the sample. Silver and yellow/silver eel population structure is dominated by the 300- to 420-mm size-class, which represents 89% of this category in both years. This size-class is dominated by males (Tesch 1977; Rossi and Colombo 1979; Adam 1997). The proportion of males was 88.9% in 1996

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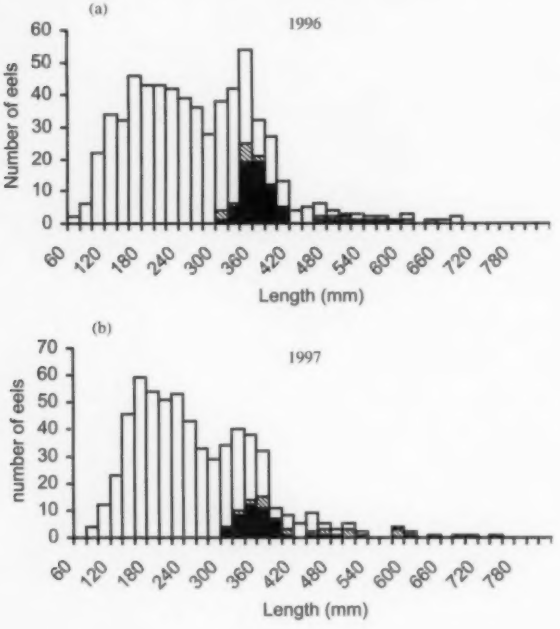
Fig. 2. Length histogram of eels sampled in the Frémur catchment in (*a*) September 1996 and (*b*) September 1997. Open bars, yellow eels (N = 531 in 1996 and N = 538 in 1997); solid bars, silver eels (N = 68 in 1996 and N = 47 in 1997); hatched bars, yellow/silver eels (N = 17 in 1996 and N = 24 in 1997).



and 89.3% in 1997. Silver and yellow/silver eels represented 13.9% of the sample in 1996 and 11.6% in 1997. There seems to be a decrease in the silver eel proportion in the samples during the study period. However, this phenomenon may be biased by the sampling methodology and needs to be verified by other means (see Population size estimates section above).

During the study period, the densities of all stages remained stable (Table 1), with an average of 0.45 eel·m^{-2}. The proportion of silver eels remains stable, with 8.7% in 1996 and 8.9% in 1997. The proportion of yellow/silver eels did not vary significantly either (2.2% in 1996 and 1997). Therefore, the proportion of potential breeders (silver and yellow/silver eels) amounts to roughly 11% of the stock in both 1996 and 1997.

The average biomass decreased from 21 g·m^{-2} in 1996 to 17 g·m^{-2} in 1997. This decrease was mainly due to yellow eels dropping from 15 g·m^{-2} in 1996 to 11 g·m^{-2} in 1997. None of these changes were statistically significant (Student t test, p = 0.30). Silver eel biomass represented 5 g·m^{-2} (23.1% of the stock) in 1996 and 4 g·m^{-2} (23.5% of the stock) in 1997, these differences being statistically insignificant (Student's t test, p = 0.31).

In 1996, a total of 59 silver eels, 17 yellow/silver eels, and 328 yellow eels were PIT tagged (Table 2). In 1997, 22 silver eels were caught (eight had already been PIT tagged as yellow or silver eels in 1996 and the remaining 14 were PIT tagged), 14 yellow/silver eels were tagged (one had been tagged the year before), and 106 yellow eels (19.7%) were tagged (48 had been tagged the year before).

Catadromous migrations

General characteristics

A total of 1333 eels (204 kg) were caught in the downstream migration trap (Fig. 3). The number of emigrants was stable during both seasons: 678 (85 kg) in 1996 and 655 (119 kg) in 1997. The biomass appeared to be 40% higher in 1997 despite a reduced number of eels, indicating an increase in the sizes in 1997 (see below). A very large majority of individuals were silver eels (99%), and only about 1% were yellow/silver eels. During the 1996 season, 2.2% of the population were yellow/silver eels and none were yellow/silver eels in 1997.

The population structures were very similar in the 1996 and 1997 seasons, with size ranges from 290 to 991 mm. The length–frequency appears to be bimodal (Fig. 3): 300–420 and >420 mm in both years. The first mode represents 73.4% of the population in 1997 versus 80.0% in 1996. All eels in this size-class are assumed to be males and those >420 mm to be females (Tesch 1977; Rossi and Colombo 1979; Adam 1997). Therefore, the emigrating eel population appeared to be dominated by males in the Frémur catchment during the study period.

Migration dynamics

Downstream migrations occurred during the whole study period (Fig. 4), but 84.9% of the migration fluxes occurred within 7 weeks. Two peaks were observed: the first lasted 4 weeks starting in February 1997, comprising 88.3% of "1996 season" migrants, and the second lasted 3 weeks starting in April 1998, representing 81.4% of "1997 season" migrants. Both migration peaks occurred very late in the season compared with usual migration periods occurring in the region and were triggered mainly by high water discharges.

Most downstream migration only occurred when the Bois Joli reservoir was full, allowing water to flow over the dam (Fig. 4). Acou et al. (2000) showed that once the water flowed over the dam, other environmental factors such as flow, atmospheric pressure, rainfall, and lunar phase (Fig. 5) influenced migration intensity. However, no significant relationship was found between temperature and migration intensity during the peak, which lasted a too short a time for this parameter to play a role (Acou 1999). A general model was developed to predict escapement of silver eels based on population parameters and environmental conditions (Acou 1999; Acou et al. 2000).

Stock – downstream migration relationship

Stock estimates from electrofishing

During the study period, electrofishing was used to assess the resident stock of eels, including silver and yellow/silver eels (Table 1). These eels were assumed to be "candidates for emigration" during the next season. According to stock estimates based on electrofishing, a total of 3000 silver eels and 750 yellow/silver eels were expected in the downstream trap during the 1996 and 1997 seasons (Table 1). But in fact, the emigrant silver eel run amounted to 678 silver and

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Table 1. Characteristics of the sedentary fraction of the eel population in 1996 and 1997 estimated by electrofishing.

	All stages	Silver eels	Yellow eels	Yellow/silver eels
1996 (N = 30)				
Density (no.·m^{-2})				
Mean	0.46	0.04	0.41	0.01
SD	0.85	0.08	0.81	0.02
Stock (no.)	34 500	3000	30 700	750
Biomass (g·m^{-2})				
Mean	21	5	15	1
SD	46	9	42	3
Stock (kg)	1 575	375	1 125	75
1997 (N = 29)				
Density (no.·m^{-2})				
Mean	0.45	0.04	0.4	0.01
SD	0.43	0.07	0.41	0.02
Stock (no.)	33 750	3000	30 000	750
Biomass (g·m^{-2})				
Mean	17	4	11	2
SD	17	8	10	4
Stock (kg)	1 275	300	825	150

Note: Stock is the estimation of the population size in the whole catchment in numbers and in kilograms (mean × water surface of the catchement) and N is the number of sampled stations.

Table 2. Number of PIT-tagged and recaptured eels marked in 1996 and 1997 using electrofishing and fyke nets.

Tagging			Recapture								
			Silver eels				Yellow silver eels		Yellow eels		
Eel stage	Electrofishing period	No.	Trap 1996	Trap 1997	EF spring 1997	EF autumn 1997	EF spring 1997	EF autumn 1997	EF spring 1997	EF autumn 1997	Total
Silver	Autumn 1996	59	12	5		2			1		20
	Spring 1997										0
	Autumn 1997	22		5							5
Yellow/silver	Autumn 1996	17				1				1	2
	Spring 1997										0
	Autumn 1997	14									0
Yellow	Autumn 1996	328	1	2		3			6	33	45
	Spring 1997					1		1		14	16
	Autumn 1997	106				1					1
	Total	546	13	12	0	8	0	1	7	48	89

Note: The eels were recaptured either in the catchment (EF) or in the traps (Trap).

yellow/silver eels during the 1996 season and 655 silver eels during the 1997 season.

Comparison of population structures

Population structures of silver eels sampled in the catchment (electrofishing and fyke nets) were very similar to those of migrant eels captured in the downstream trap (Fig. 3). A very slight difference in the size of males occurred, with those of the catchment being 15 mm smaller on average than those caught in the trap. Also, females were slightly less represented in the catchment than in the trap. However, none of the observed differences were statistically significant, according to Student's t and Kolmogorov–Smirnov tests ($p > 0.95$), respectively. Therefore, both approaches gave a very similar representation of silver eels population in the catchment, and since the traps constitute an exhaustive method, we assume that the sampling plan that we used provides for reliable information on silver eel populations at the scale of the river system.

Stock estimates using PIT tags

Among the 59 silver eels tagged in 1996, 12 in 1996 and five in 1997 were found in the downstream trap (Table 2). In other words, 20.3 % of the candidates for emigration (silver eels) present in the catchment actually emigrated during the following migration season. The stock of silver eels in the total catchment is estimated (Petersen estimate) at 3340 in 1996 and 2885 in 1997. These results are very similar to those obtained by electrofishing (see Table 1), suggesting that probably both stock assessment methods are efficient.

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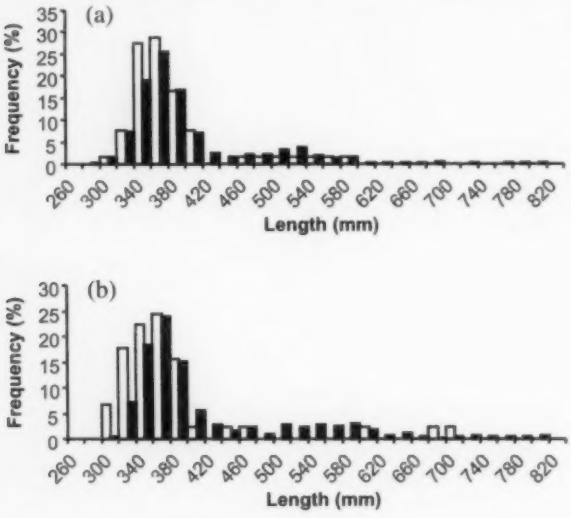
Fig. 3. Comparison of silver eel population structure caught in the catchment (river) by electrofishing and fyke nets in (*a*) September 1996 and (*b*) September 1997 and the emigrating silver eel population (descending) caught in the downstream trap during the subsequent 1996 season (October 1, 1996, to September 9, 1997) and 1997 season (October 1, 1997, to June 1, 1998). Open bars, silver eels caught in the river (68 and 47 eels in 1996 and 1997, respectively); solid bars, silver eels caught in the downstream trap (678 and 655 in 1996 and 1997, respectively).



Migrating behaviour and mortality

Among the 59 silver eels tagged in 1996, 12 (20.3%) were found in the downstream trap during the 1996 season (Table 2). A total of three were recaptured in the river in 1997; among them, two were still silver eels and one had lost silver eel characteristics. During the 1997 season, five of the eels tagged in 1996 were caught in the downstream trap. Combined with the two caught in the river at silver eel stages, at least seven eels (11.9%) remained silver during more than a year and five (8.5%) emigrated more than 18 months after metamorphosis into silver eels. This means that 20 eels (33.9%) were recaptured, from which 17 (28.8%) were recaptured in the downstream trap. At the end of the experiment, the remaining eels were still in the catchment, died, or escaped during floods. Among the 22 silver eels tagged in 1997, only five (22.7%) were recaptured during the following migration season.

Among the 17 yellow/silver eels tagged in 1996, two were recaptured in the catchment during autumn 1997, one as a silver eel and the other as a yellow eel. None of the yellow/silver eels marked in 1996 and in 1997 were recaptured in the downstream trap the following year. This suggests that they are in a maturing stage (or regression, since one had been found as a yellow/silver eel). Therefore, they do not appear to be real candidates for emigration.

A total of 328 yellow eels were tagged in 1996. One was captured the same year as a silver eel in the downstream trap, suggesting a rapid metamorphosis. Two were caught in the downstream trap during the following season. In the catchment, 42 eels were recaptured, including 39 yellow eels (93%) and three silver eels (7%). In autumn 1997, 106 yellow eels were tagged. One was recaptured a few days later.

Discussion

The present study permitted the characterisation of the eel stock in the Frémur catchment, showing that silver eels represented almost 10% of the sedentary population. We assume that this percentage is representative of short and obstructed rivers, but extrapolation to other systems should be considered carefully according to local environmental conditions and population parameters.

The sampling was conducted in September in both years, when most silvering eels have already started or finished their metamorphosis (Tesch 1977; Fontaine 1994). Therefore, it was possible to obtain reliable information on the size (3000 silver eels), length ratio, and sex ratio (85% males) of the silver eel population in the whole river system. The silver eel stock present in the river before emigration was estimated at 3340 in 1996 and 2885 in 1997. Results were similar to those obtained by electrofishing (3000 silver eels in both years), suggesting that both methods are reliable.

The characteristics of the resident stock were compared with downstream migration data collected at the trap. The basic question was "is it possible to predict the downstream migration fluxes from quantitative data about the sedentary fraction of an eel population and from environmental parameters?"

Around 20% of the eels identified as silver eels migrated during the following year, as soon as favourable environmental conditions occurred. The quite stable ratio that was observed in both years is probably due to stable population parameters and to favourable migration conditions that occurred sometime in both years. At least 5% stayed in the river where they were found the next year during electrofishing and 1.5% recovered yellow eel characteristics. Recovery of yellow eel characteristics has previously been described (Lee 1979). Almost 9% of the eels stayed an extra year in the catchment and finally emigrated. Therefore, the fate of the 66% of the silver eels that were not recaptured is unknown. Several hypotheses can be postulated. Firstly, our sampling effort failed to recapture the marked eels (only 2.5% of the river length sampled), suggesting that they were still dwelling somewhere in the river; subsequent sampling campaigns should enable us to understand the fate of these eels. Secondly, eels may escape from the downstream trap during the migration peak. Roughly 37% of escapement occurred when the flow exceeded 1500 $L \cdot s^{-1}$; some of the tagged eels may have escaped then, but this only concerns at most 2% of the silver eels. Thirdly, the silver eel stage was not defined correctly by macroscopic criteria, suggesting that migration behaviour is acquired at the end of the silvering metamorphosis, which itself probably lasts several months. This is confirmed by the fact that none of the yellow/silver eels were found in the downstream traps. It also indicates that a silver eel index is needed to correctly assess the size of the "migration candidate" population. Fourthly, natural mortality might have reduced the tagged population. Usually, high eel mortality is rather low in "natural populations," about 5–10%·$year^{-1}$ (e.g., Adam 1997). In

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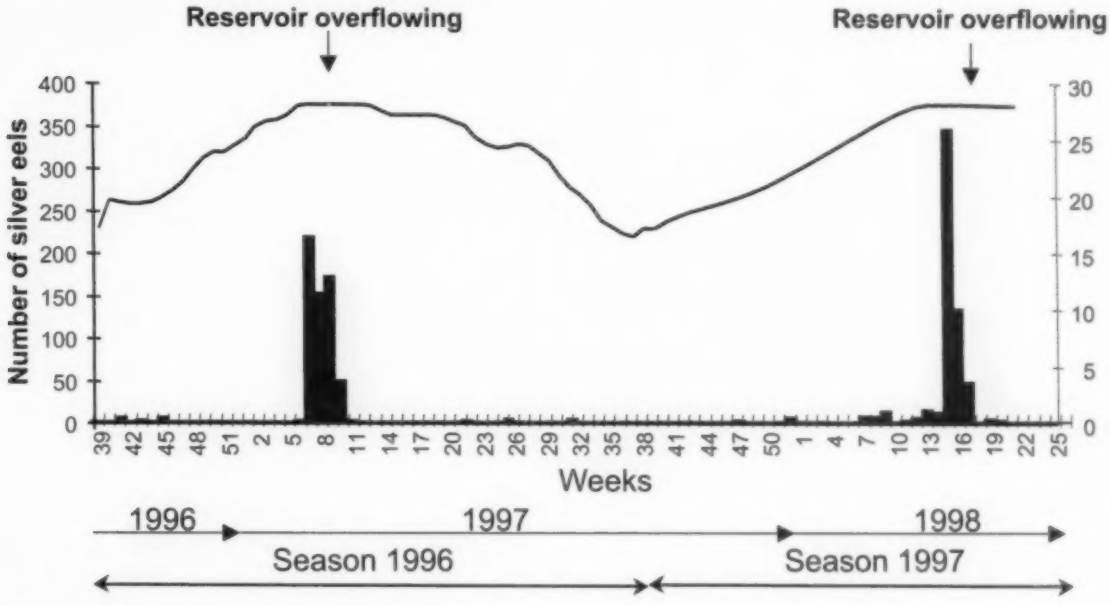
Fig. 4. Weekly trends of silver eel emigration intensity between October 1996 and June 1998 and corresponding water levels in Bois Joli Lake located 6 km from the sea. Bars, weekly catch in the trap; lines, water level in the reservoir.



the Frémur, fishing pressure is low (no professional fishery, while anglers mainly focus on cyprinids, esocids, or percids), predation mortality is low because there are few cormorants or herons, and no massive mortality related to eutrophication has been observed (E. Feunteun, unpublished data). Lastly, anthropogenic mortality due to the management of Bois Joli dam has to be considered. Emigration is only possible once the reservoir is full and the water spills over the discharge weirs. The rest of the time, a minimal water flow is controlled by pipes. Emigrant silver eels are probably forced to go through them, but they are killed by pressure at the outlet. A study has just begun to determine this mortality factor. Preliminary results show that about 12% of the descending silver eels are probably killed because of the pipes (E. Feunteun et al., unpublished data). This is consistent with mortality caused by passage through turbines, which ranges between 5 and 100% according to Berg (1987) and Langon and Dartidelongue (unpublished).[2]

The current study confirmed the influence of various environmental parameters (river discharge, rainfall, atmospheric pressure, temperature, moon phase, etc.) on the emigration dynamics (e.g., Tesch 1977; Haraldstad et al. 1985; Vollestadt et al. 1986). However, the influence of dam management overruled environmental factors: emigration was impossible until the reservoir was full and water overflowing permitted eels to slide down the 14-m-high weir. The reservoir overflowed quite late in both seasons (February 1997 and April 1998), provoking important delays in the migration peaks compared with nonobstructed rivers (Tesch 1977; Vollestadt et al. 1986). Although silver eels are known to delay emigration by up to a whole year until favourable conditions occur (Vollestadt et al. 1994), the consequences of such a delay on breeding success remain unknown. We assume that if the silver eels do not feed during that period, their fat content will obviously diminish, compromising migration and breeding success. The obstructions due to hydraulic works on downstream migration have rarely been looked at, despite the probably important damage that they cause to the eel stocks by reducing breeder escapement from continental waters (Fontenelle et al. 1997). Once the dam was full, downstream migration was triggered by flow increases. After the flow peak, rainfall and (or) low atmospheric pressure may have influenced the migration intensity.

In conclusion, the results of our study were obtained during a period that was too short to provide for a reliable model of the stock–emigration relationships. However, this approach needs to be conducted at least on the mean term to see the effects of unfavourable environmental conditions on migration dynamics. Indeed, during the study period, the conditions permitted emigration, even if a delay in the migration peaks was observed. But how would the silver eel population react to dry weather conditions as observed between 1989 and 1991?

The Frémur is very much like a wide range of small catchments of western France, and therefore, the results could eventually be extended to define a regional escapement index without actually measuring escapement everywhere. However, downstream migration dynamics probably vary according to the catchment's size, habitat index, geographic situation, etc., since such parameters are known to influence sex ratio, population structure, and size. Thus, such an approach needs to be conducted at the European and

[2] Langon, M., and Dartidelongue, J. 1997. La dévalaison des anguilles (*Anguilla anguilla* L.) argentées — test de deux dispositifs de dévalaison à la centrale hydroélectrique EDF d'Halsou (Nive, Pyrénées Atlantiques), 1996. Revue bibliographique.

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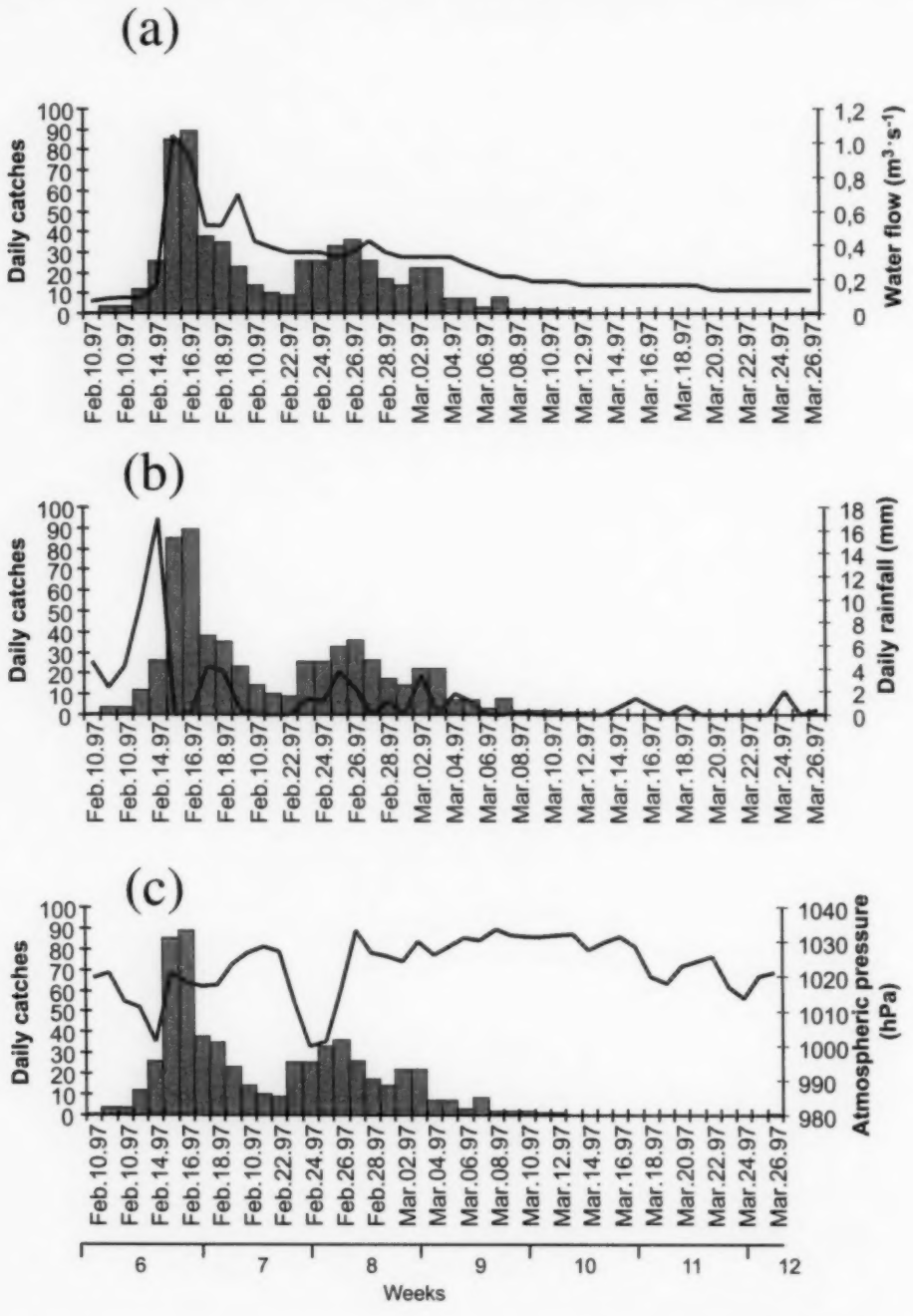
Fig. 5. Emigration intensity and water flow during the migration peak in the 1997 season (N = 599 silver eels). Bars, daily catches; lines, environmental measures: (*a*) water flow, (*b*) rainfall, and (*c*) atmospheric pressure.



North African level to choose a range of representative aquatic continental systems and to define a European silver eel breeder escapement index based on stock characteristics and extrinsic environmental parameters. Such a methodology is required to start reliable monitoring of the species, as recommended by ICES/EIFAC working group on eels (Moriarty and Dekker 1997). This is the first step in defining a stock (breeder escapement) – recruitment (subsequent glass eel immigration) relationship, which is needed to develop a reliable sustainable management policy for the spe-

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cies. Moreover, this kind of approach is important in understanding the implementation of management measures to mitigate impact of dams on silver eel escapement from riverine systems.

Acknowledgements

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Suitability criteria analyzed at the spatial scale of redd clusters improved estimates of fall chinook salmon (*Oncorhynchus tshawytscha*) spawning habitat use in the Hanford Reach, Columbia River

David R. Geist, Julia Jones, Christopher J. Murray, and Dennis D. Dauble

Abstract: We improved our predictions of fall chinook salmon (*Oncorhynchus tshawytscha*) habitat use by analyzing spawning habitat at the spatial scale of redd clusters. Spatial point pattern analyses indicated that redd clusters in the Hanford Reach, Columbia River, were consistent in their location from 1994 to 1995. Redd densities were 16.1 and 8.9 redds·ha^{-1} in 1994 and 1995, respectively, and individual redds within clusters were usually less than 30 m apart. Pattern analysis also showed strong evidence that redds were uniformly distributed within the clusters where interredd distances ranged from 2 to 5 m. Redd clusters were found to occur predominantly where water velocity was between 1.4 and 2 m·s^{-1}, water depth was 2–4 m, and lateral slope of the riverbed was less than 4%. This habitat use represented a narrower range of use than previously reported for adult fall chinook salmon. Logistic regression analysis determined that water velocity and lateral slope were the most significant predictors of redd cluster location over a range of river discharges. Overestimates of available spawning habitat lead to nonachievable goals for protecting and restoring critical salmonid habitat. Better predictions of spawning habitat may be possible if cluster-specific characteristics are used.

Résumé : Nous avons amélioré nos prédictions de l'utilisation de l'habitat par le quinnat (*Oncorhynchus tshawytscha*) d'automne en analysant l'habitat de fraye à l'échelle spatiale des grappes de nids. Les analyses de voisinage ont indiqué que les grappes de nids dans le tronçon Hanford du fleuve Columbia étaient localisées de façon uniforme de 1994 à 1995. Les densités étaient respectivement de 16,1 et 8,9 nids à l'hectare en 1994 et 1995, et, à l'intérieur des groupements, les nids étaient généralement distants de moins de 30 m. L'analyse de voisinage a aussi montré nettement que les nids étaient distribués de façon uniforme à l'intérieur des grappes, les distances entre les nids allant de 2 à 5 m. Les grappes de nids se retrouvaient principalement aux endroits où la vitesse de l'eau se situait entre 1,4 et 2 m·s^{-1}, la profondeur de l'eau était de 2 à 4 m, et la pente latérale du lit de moins de 4%. Cette utilisation de l'habitat représentait une plage plus étroite que ce qui avait été signalé antérieurement pour le quinnat d'automne adulte. L'analyse de régression logistique a fait ressortir que la vitesse de l'eau et la pente latérale étaient les prédicteurs les plus significatifs de l'emplacement d'une grappe de nids pour une série de débits du cours d'eau. Si l'habitat de fraye disponible est surestimé, les buts fixés pour la protection et la restauration de l'habitat critique des salmonidés ne pourront pas être atteints. Il serait possible d'avoir de meilleures prédictions pour l'habitat de fraye si on s'appuie sur les caractéristiques particulières aux grappes de nids.

[Traduit par la Rédaction]

Introduction

Physical habitat simulation models (PHABSIM; Milhous 1979; Stalnaker 1979) are used in instream flow assessment studies to evaluate how spawning habitat of salmon changes with varying stream flow (Shirvell 1989; Connor et al. 1990, 1994). PHABSIM combines physical characteristics of the river channel (e.g., substrate and channel dimensions) into a hydraulic model that simulates how water surface elevation, depth, and velocity will change over a range of river discharges (Bovee and Bartholow 1995). A "map" of the river channel can be generated that provides a picture of what the physical characteristics of the river would look like at each simulated river discharge. To translate this picture into an estimate of salmon spawning habitat, spawning habitat suitability criteria are used to define the ranges of depths, velocities, and substrates that are utilized by spawning salmon. The physical attributes of the river are compared against the habitat suitability criteria to determine the relative value of physical characteristics for spawning habitat.

The standard procedure when using PHABSIM to predict available spawning habitat is to collect or use spawning habitat criteria that originate within the river of interest (Bovee 1995). River-specific suitability criteria were used in a previous application of PHABSIM to fall chinook salmon

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D.R. Geist[1] **and D.D. Dauble.** Ecology Group, Pacific Northwest National Laboratory, P.O. Box 999, MS K6-85, Richland, WA 99352, U.S.A.
J. Jones. Geosciences Department, Oregon State University, Corvallis, OR 97331, U.S.A.
C.J. Murray. Applied Geology and Geochemistry Group, Pacific Northwest National Laboratory, P.O. Box 999, MS K6-81, Richland, Washington, 99352, U.S.A.

[1]Author to whom all correspondence should be addressed. e-mail: david.geist@pnl.gov

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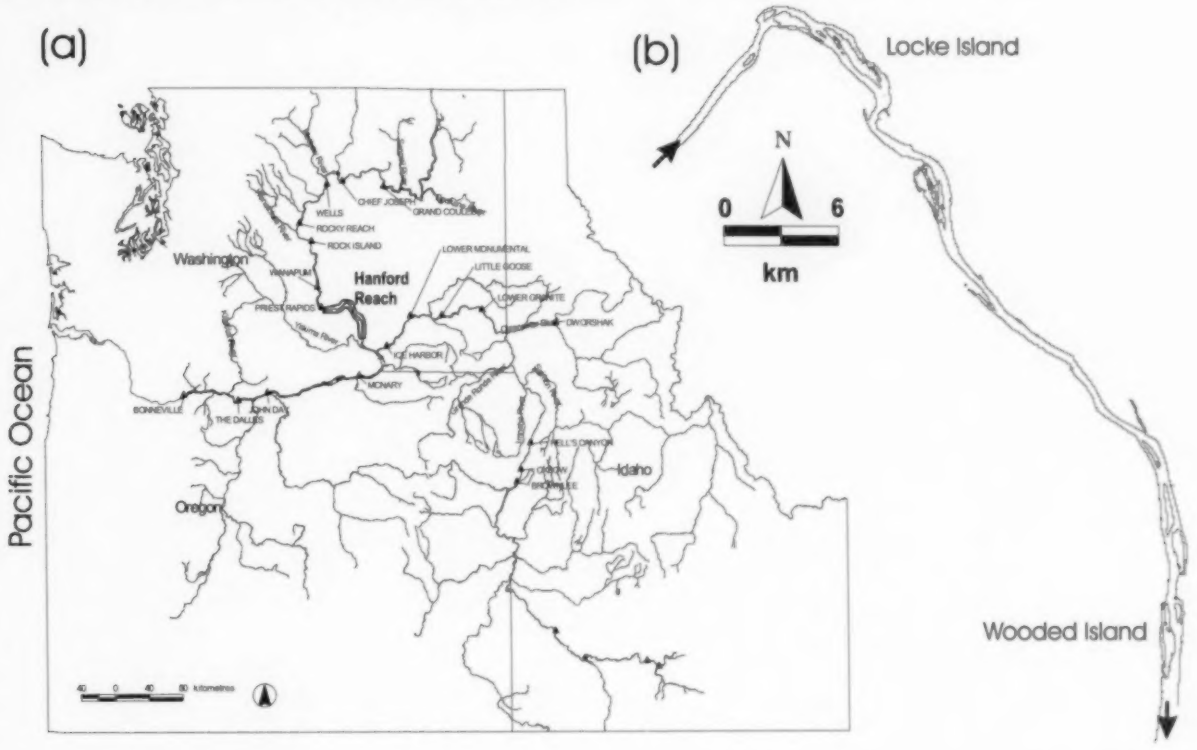
Fig. 1. (*a*) Pacific Northwest of the United States with the Hanford Reach of the Columbia River highlighted. Mainstem hydroelectric dams (triangles) and major tributaries are also shown. (*b*) Enlargement of the Hanford Reach showing the Locke Island and Wooded Island study sites. Arrows indicate the flow direction of the river.



(*Oncorhynchus tshawytscha*) spawning habitat in the Hanford Reach of the Columbia River (Geist et al. 1997). In that study, approximately 200 redds were randomly sampled from spawning areas located throughout the entire length (~80 km) of the Hanford Reach. Fall chinook salmon redds were found to occur in water velocities that ranged from 0.5 to 2.0 $m \cdot s^{-1}$, water depths of 0.5–7.0 m, and substrate particles that were 2.5–15.2 cm in diameter (Geist et al. 1997). This was consistent with habitat use by adult fall chinook salmon in other parts of the Columbia River Basin (reviewed by Geist and Dauble 1998), including the Snake River where fall chinook salmon are listed under the Endangered Species Act (Groves and Chandler 1999). Using these criteria, PHABSIM predicted that approximately 50% of the river bottom at each of two spawning areas in the Hanford Reach could have been utilized for spawning. In contrast, even though fall chinook salmon escapements during the study were sufficient to completely seed the two areas (D.R. Geist, unpublished data), actual use was 0% at one area and 20% at the second area.

We believe that the disparity between PHABSIM predictions and actual use occurred because the suitability criteria did not consider that fall chinook salmon redds are typically aggregated in patches or "clusters" within the Hanford Reach (Geist and Dauble 1998). The patchy redd distribution provides strong evidence that clusters may contain specific features that fall chinook salmon use when selecting spawning locations. We hypothesized that the PHABSIM estimates from Geist et al. (1997) would have been improved had they incorporated habitat suitability criteria from redd clusters rather than from individual redds that were sampled throughout the reach.

The objective of this paper was to determine if an analysis of suitability criteria based on cluster-scale measurements would result in better predictions of habitat use from two spawning areas where PHABSIM previously overestimated spawning habitat (Geist et al. 1997). We applied spatial point pattern analysis to demonstrate that spawning was clustered and logistic regression analysis to identify important factors influencing redd distribution. Our intent is to apply the improved predictions of habitat use by adult fall chinook salmon to develop recovery plans for fall chinook salmon from the Columbia River Basin.

Materials and methods

Study area

The Hanford Reach of the Columbia River is approximately 80 km long, unimpounded, and bounded by Priest Rapids Dam on the upstream end and McNary Reservoir on the downstream end (Fig. 1*a*). The largest population of fall chinook salmon in the Columbia River Basin returns each year to spawn in the Hanford Reach (Dauble and Watson 1997). Peak fall chinook salmon

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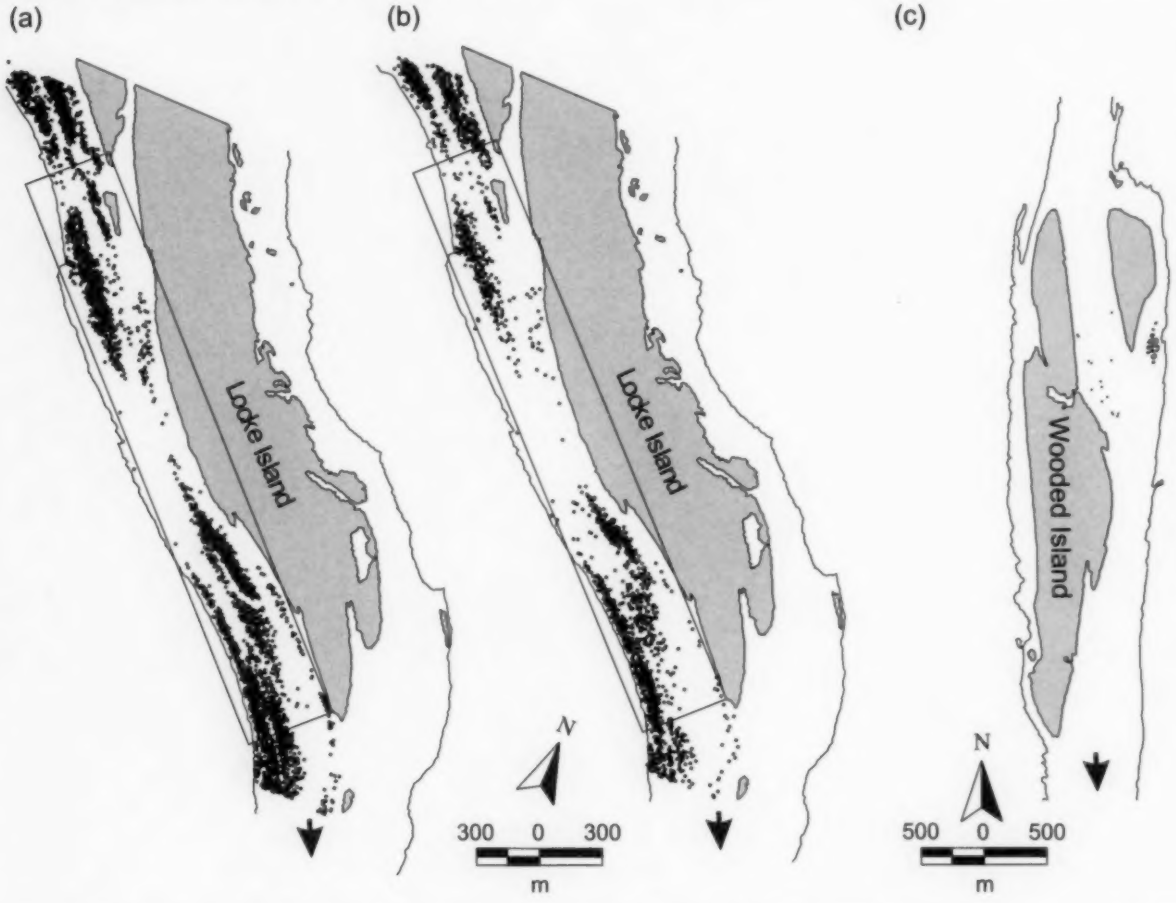
Fig. 2. Distribution of fall chinook salmon redds following peak spawning at (*a*) Locke Island in 1994, (*b*) Locke Island in 1995, and (*c*) Wooded Island in 1995. The rectangular boundary where the spatial pattern analysis was completed is shown in Figs. 2*a* and 2*b*. In each panel, an arrow indicates the flow direction of the river.



spawning occurs from approximately the last week in October through the third week in November (Dauble and Watson 1990).

Physical habitat data were collected at two sites in the Hanford Reach (Fig. 1*b*): Locke Island (river kilometres 596–600) and Wooded Island (river kilometres 560–563). Each study site was approximately 3.5 km in length and 300–400 m wide and occurred in a braided channel. These sites have similar channel characteristics but very different redd densities. The average annual redd count from 1990 to 1995 was 513 redds (range 340–855 redds) and 10 redds (range 0–32 redds) for the Locke Island and Wooded Island study sites, respectively (Dauble and Watson 1997; D.D. Dauble, unpublished data).

Although the Hanford Reach is unimpounded, upstream hydropower dams control flow through the study sites. During the fall chinook salmon spawning period, flows at Priest Rapids Dam are operated according to the Vernita Bar Settlement Agreement (Federal Energy Regulatory Commission 1988). The primary objective of the Agreement is to minimize spawning in areas where flow cannot be maintained throughout the egg incubation period. This is accomplished through reverse load factoring, i.e., influencing the location of fall chinook salmon redds by intentionally reducing power generation during the daylight hours (approximately 06:00–17:00) and then increasing generation during hours of darkness. Implicit in the Agreement is that fall chinook salmon are capable of completing spawning within a 24-h period between two successive nighttime flows (Chapman et al. 1986).

Spatial pattern analysis

Fall chinook salmon redds were photographed at each site from a fixed-wing airplane flying approximately 600 m above the water surface (photographic scale of ~1:2400). Flights occurred during the peak spawning interval with one flight per week made in 1994 (Locke Island site only) and 1995 (both sites). Each redd was digitized into an Arc-Info® geographic information system. Cumulative distributions for week 4 of the spawning period are presented here (Fig. 2). Aerial photographs were limited by water depth. However, additional redd surveys, conducted in 1995 using an underwater video camera, showed that no additional redds were present outside the area of photographic record.

Refined nearest-neighbor analysis (Boots and Getis 1988) was used on the digitized redds to determine whether fall chinook salmon redds were randomly distributed or if they followed a uniform (i.e., regular) and (or) clustered pattern. The spatial pattern analysis was also used to determine the distance between redds within any given pattern type. The nearest-neighbor analysis required the use of a rectangular boundary for the study area. For

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that reason, a reasonably straight section of the river within the Locke Island study site was chosen, and the location coordinates of the redds were transformed by rotation so that the long axis of the spatial study area was parallel to the longitudinal axis of the river (Fig. 2). This provided a rectangular boundary 425 m wide and 2 850 m long. A contingency table and χ^2 test were also used in that study area to test the hypothesis that occupation of a 20 × 20 m cell by a chinook salmon redd in 1995 was independent of occupancy in 1994.

Refined nearest-neighbor analysis (Boots and Getis 1988) uses the cumulative distribution function $F(d)$ to describe the probability that the nearest neighbor to a redd is within a given distance d. For a random spatial distribution generated by a Poisson process, the expected cumulative distribution function is

$$F(d) = 1 - e^{-\lambda \pi d^2}, \; d \geq 0$$

where λ is the intensity of the points within the area, estimated by $\lambda = n/A$ for n points in area A. The empirical cumulative distribution of distances can be calculated from the data set for each distance d and compared with the expected value for that distance.

The software used to conduct the spatial pattern analyses (Moeur 1993) generates a Monte Carlo confidence envelope around the expected value for each distance d. The empirical cumulative distribution determined from the data is compared with the confidence envelope for each d: if the proportion of the nearest neighbors less than distance d is outside the confidence envelope, then the hypothesis that the spatial pattern of the data points resulted from a random process is rejected at the 95% confidence level. The direction of the deviation above or below the confidence envelope indicates whether the nonuniform pattern tended toward a clustered or uniform distribution, respectively.

Hydraulic habitat data

Hydraulic data were collected following the procedures of the instream flow incremental methodology (Bovee 1982). Eight transects were set perpendicular to the flow, approximately 450 to 550 m apart, at each study site. River stage and river discharges were measured on three separate occasions (October 1995, June 1996, and July 1997) to establish stage–discharge relationships. Elevations were measured using a laser transit (Leitz/Sokkisha Set 2 Electronic Total Station®). Discharge was measured using an acoustic Doppler current profiler (ADCP model 600®). River discharge during the surveys ranged from 2 206 to 7 061 $m^3 \cdot s^{-1}$.

During hydraulic calibration surveys conducted in October 1995, mean water column velocities, channel elevations, and substrate sizes were measured at locations along each transect, which we refer to as "habitat cells" (Payne and Lapointe 1997). Habitat cells were assumed to represent depth and velocity conditions of a hydraulically uniform area of river. An ADCP was used to measure water velocity and depth along each transect when water depth was greater than 1 m. At water depths less than 1 m, channel elevation and water velocity (0.6 of the water depth) were measured using a standard top-set wading rod and a Marsh McBirney® flowmeter. Lateral slope of the river bottom at each habitat cell was determined using cross-section elevations of the transects and water depths measured with the ADCP. Average river discharge at the Locke Island and Wooded Island study sites during these surveys was 1950 and 2462 $m^3 \cdot s^{-1}$, respectively.

Substrate measurements were made on the hydraulic transects and on four additional transects that overlapped and bounded the spawning areas. Images of the substrate on the surface of the riverbed were collected using an underwater camera suspended about 40 cm above the riverbed (Garcia et al. 1994; Groves and Garcia 1998). Up to 20 substrate images were collected on each transect, or approximately 250 images at each study site. The location of substrate images was recorded using a global positioning system (Trimble Pro-Excel®). All individual substrate clasts within each image were measured (long-axis diameter and surface area) and stored using a computer program (Optimus®). Each substrate image was assigned a dominant and subdominant size-class (Brusven 1977; Groves and Chandler 1999) based on long-axis diameter. The percentage of fine-grained material ($d < 2$ mm) visible within interstitial spaces was also recorded.

We used IFG-4 (Milhous et al. 1984) to simulate depth and velocity at each habitat cell. Simulation flows were selected to represent the range of flows under which fall chinook salmon spawn. We excluded nighttime flows from our analysis because they were intentionally high due to reverse load factoring and artificially raised the average flows above levels that we believed would be representative of potential spawning flows. Flow fluctuations at Priest Rapids Dam are attenuated with increasing distance downstream and not representative of the minimum or maximum hourly flows that occurred at the study sites. This has been verified by comparing hourly discharge at Priest Rapids Dam with continuous stage measurements of the river recorded at hourly increments (Walters et al. 1994). Therefore, hourly flows from Priest Rapids Dam were input to a one-dimensional hydraulic simulation model in order to estimate the minimum (Q_{min}), average (Q_{ave}), and maximum (Q_{max}) hourly flows during the daylight hours (06:00–17:00) within the peak spawning period at the Locke Island (1994 and 1995) and Wooded Island (1995 only) study sites (Fig. 3). The Q_{min}, Q_{ave}, and Q_{max} flows at the Locke Island site were 1453, 2034, and 3034 $m^3 \cdot s^{-1}$, respectively, in 1994 and 1896, 2474, and 3481 $m^3 \cdot s^{-1}$, respectively, in 1995. The Q_{min}, Q_{ave}, and Q_{max} flows at the Wooded Island site in 1995 were 2285, 2974, and 3420 $m^3 \cdot s^{-1}$, respectively. Based on the close agreement between simulated river stage and actual river stage (less than ±10 cm), flow simulations were estimated to be within less than ±5% of the actual values (G. Guensch, Pacific Northwest National Laboratory, Richland, Wash., personal communication).

Velocity adjustment factors were calculated by dividing the simulated velocity by the measured velocity (Bovee and Bartholow 1995). Simulations were rejected if the velocity adjustment factor was less than 0.1 or greater than 5.0; none of our simulations exceeded these criteria. We used measured values for substrate and lateral slope, as we assumed that these values would not change between the simulated flows and measured flows. Following hydraulic simulations, each habitat cell contained a value for depth, substrate class (dominant and subdominant), mean water column velocity, and lateral slope.

Logistic regression model

Logistic regression, based on negative log likelihood (Hosmer and Lemeshow 1989), was used to determine which explanatory variables were important in spawning habitat selection. The specific form of the logistic regression model was

$$\pi(x) = \frac{e^{g(x)}}{1 + e^{g(x)}}$$

where $\pi(x)$ was the probability that a habitat cell would be suitable for spawning and $g(x)$, the logit transformation of $\pi/(1 - \pi)$ (Hosmer and Lemeshow 1989), was the linear combination of parameter estimates from the logistic regression

$$g(x) = B_0 + B_1X_1 + \ldots + B_nX_n.$$

A logistic model is appropriate because it can be used to examine the functional relationship between a binary response (suitable versus unsuitable) and explanatory variables that describe the quality of the habitat. The model estimates the probability of a positive response occurring given a set of explanatory variables (Hosmer and Lemeshow 1989). It has been used extensively in retrospective

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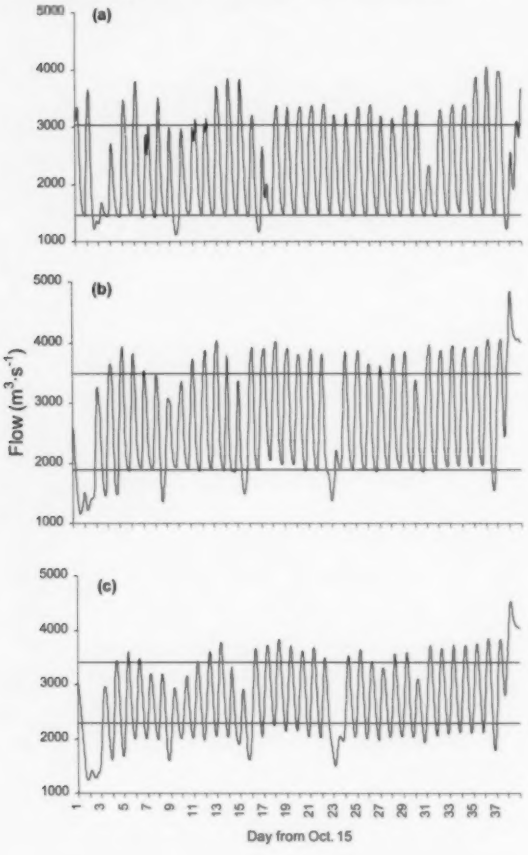
Fig. 3. Simulated hourly discharge of the Columbia River at the Locke Island study site in (*a*) 1994 and (*b*) 1995 and (*c*) at the Wooded Island study site in 1995. Simulations were performed using a one-dimensional hydraulic model that used actual flow data from Priest Rapids Dam but compensated for flow attenuation between the dam and each study site. In each panel, the upper and lower lines represent the daily average maximum and minimum hourly discharges, respectively, that occurred during the peak spawning period (October 15 – November 21).



studies of habitat use by various species of fish (e.g., Rieman and McIntyre 1995; Wiley et al. 1997; Torgersen et al. 1999).

Fall chinook salmon spawning habitat suitability was the binary response variable (suitable or unsuitable) for the logistic model. We used the results of the spatial pattern analysis to determine whether habitat cells fell inside or outside redd clusters. Habitat cells that fell inside redd clusters were assumed to be representative of "suitable" spawning habitat and were coded as 0. Habitat cells that fell outside the boundaries of the clusters were assumed to be representative of "unsuitable" spawning habitat and were coded as 1. Depth, velocity, substrate (dominant and subdominant), and lateral slope values from each habitat cell were treated as explanatory variables. All explanatory variables were treated as continuous variables with the exception of the variables dominant substrate and subdominant substrate, which were both treated as discrete factors with six levels based on substrate diameter: 1 = <0.63 cm, 2 = 0.63–2.54 cm, 3 = 2.55–5.08 cm, 4 = 5.09–7.62 cm, 5 = 7.63–15.20 cm, and 6 = >15.20 cm.

The logistic regression model and parameters were derived using 1994 data from Locke Island only. A stepwise process with addition of variables was used to attain the final model (Hosmer and Lemeshow 1989). Separate variables representing depths and velocities at Q_{min}, Q_{ave}, and Q_{max} flows were used to evaluate the effect of flow variability on spawning site selection. Variables were added to the multivariate model successively if they improved model fit based on Wald's χ^2 test and the likelihood ratio test (α = 0.05; Ramsey and Schafer 1997). Once relevant variables were selected, all combinations of interaction terms and squared terms were investigated and, again, those terms that met the Wald test and the log likelihood criterion were included in the final model. The odds ratio (Hosmer and Lemeshow 1989) was used to describe the relationship between independent variables and the response variable.

The final model was used to predict habitat suitability at the Locke Island and Wooded Island sites in 1995. Predictions were compared with actual observations with separate predictions made for the Q_{min}, Q_{ave}, and Q_{max} flows estimated during 1995. A habitat cell was predicted to be suitable for fall chinook salmon spawning if $\pi(x) > 50\%$. A χ^2 test on a contingency table was used to compare the predicted designation of the habitat cell with the actual use by spawning chinook salmon.

Results

Spatial pattern analysis

There were large differences in the number of redds between Locke Island and Wooded Island (Fig. 2). Only 15 redds were recorded on the aerial photographs at the Wooded Island site in 1995, whereas redd numbers at the Locke Island site were 2481 in 1994 and 1359 in 1995. Average redd density (total redd numbers) within the rectangular box at Locke Island (Fig. 2) was 16.1 (1 947) and 8.9 (1 074) redds·ha^{-1} in 1994 and 1995, respectively. This comparison indicated a 45% reduction in the number of redds from 1994 to 1995. The locations where fall chinook salmon spawned were extremely consistent during both years (Fig. 4). For example, the number of cells occupied in 1994 and 1995 was approximately three times the expected value under random conditions ($P < 0.001$). A test of the contingency table was highly significant ($\chi^2 = 883$, df = 1), indicating a low probability ($P << 0.001$) that the reoccupation occurred by chance alone.

The spatial point patterns of chinook salmon redds at Locke Island in 1994 and 1995 were significantly nonrandom (Fig. 5). The refined nearest-neighbor analysis indicated clustering by positive deviations above the Monte Carlo confidence envelope for distances greater than 7 m and showed that the vast majority of redds were located within 30 m of another redd (>99% for 1994 and >97% for 1995) (Fig. 5). Most of the defined clusters contained at least 20 redds. In 1994, the spatial pattern analysis also indicated the presence of a nonrandom distribution of redds for short distances of 2–5 m (Fig. 5*a*). At those distances, however, the empirical distributions measured from the data fell below the confidence envelopes, indicating that those redds were more uniformly distributed than would be expected for a random process.

Hydraulic habitat data

Hydraulic data were collected at 403 habitat cells at the Locke Island study site in 1994. The pattern analysis showed

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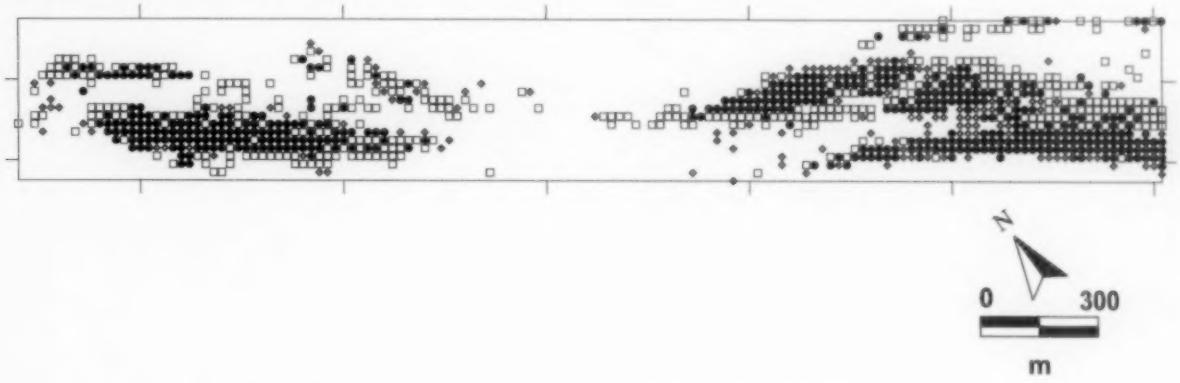
Fig. 4. Distribution of fall chinook salmon redds in 1994 and 1995 within a 425 × 2850 m area at the Locke Island study site. Depending on the distribution of redds, a cell (20 × 20 m) was coded in one of four ways: no symbol = no redds in 1994 and no redds in 1995; squares = redds present in 1994 but not in 1995; diamonds = no redds present in 1994 but redds present in 1995; circles = redds present at that location in both years.



that 84 of these cells fell within redd clusters and were defined as suitable spawning habitat. The remaining cells (319) fell outside redd clusters and were defined as unsuitable spawning habitat. Habitat cells within redd clusters had significantly lower lateral slope, shallower depths, and significantly higher velocities than habitat outside redd clusters (Fig. 6). Spawning habitat inside redd clusters also contained significantly more dominant substrate between 5.1 and 15.2 cm and subdominant substrate between 2.5 and 7.6 cm than habitat outside redd clusters (Fig. 6). Approximately 80% of the habitat inside redd clusters was characterized by water velocity between 1.4 and 2.0 $m \cdot s^{-1}$, lateral slope less than 4%, and water depth of 2.0–4.0 m. Less than 2.5% of the habitat cells inside redd clusters contained water velocities less than 1.0 $m \cdot s^{-1}$. In contrast, approximately 40% of the habitat cells outside redd clusters were characterized by water velocities less than 1.0 $m \cdot s^{-1}$.

Logistic regression model

Multivariate logistic regression analysis of the hydraulic data collected at the Locke Island site in 1994 revealed that substrate was not a significant predictor of spawning habitat suitability. This was in contrast with the univariate analysis that showed that substrate was significantly different inside and outside redd clusters (Fig. 6). However, the multivariate analysis was consistent with the results from the univariate analysis for the other hydraulic variables. Stepwise logistic regression selected lateral slope and depth, velocity, and the velocity-squared term at the Q_{ave} flow as significant variables explaining the spawning site selection by fall chinook salmon at Locke Island (Table 1). The highest odds ratios were associated with velocity, suggesting that this variable was most important in predicting spawning habitat suitability. Both lateral slope and depth were negatively associated with spawning habitat suitability. The odds ratios showed that a decrease of approximately 1% in slope resulted in a 25% increase in the probability of the site being utilized for spawning, while a decrease in depth of 0.5 m resulted in an increase in the probability that the site would be utilized for spawning of approximately 60%.

At Locke Island in 1995, the logistic regression model explained a significant proportion of the variance in habitat cell designation, with approximately 78–87% of the habitat cells correctly predicted as either suitable (ps,as) or unsuitable (pu,au) spawning habitat (Fig. 7). Even though the percentage of correct predictions was relatively high at Locke Island, 29–56% (Fig. 7, ps,au/ps) of the sites predicted to be utilized for spawning were not (i.e., errors of commission). Sites where errors of commission occurred contained similar physical features as those sites where spawning occurred, i.e., mean lateral slope, depth, and velocity of 2.4%, 2.5 m, and 1.6 $m \cdot s^{-1}$, respectively. Approximately 9–19% of spawning at Locke Island (Fig. 7, pu,as/pu) occurred in areas that the model predicted as unusable habitat (i.e., errors of omission). Sites where errors of omission occurred had a mean lateral slope of 3.5%, a water depth of 3.9 m, and a velocity of 1.8 $m \cdot s^{-1}$.

Although fall chinook salmon appeared to spawn within a specific area at Wooded Island (Fig. 2), there were an insufficient number of redds within 30 m of one another to be considered clustered. Thus, none of the habitat cells at Wooded Island were coded as suitable spawning habitat. At Wooded Island, the logistic regression model correctly predicted habitat cell designation (pu,au) 79–91% of the time, with approximately 9–21% of the habitat cells predicted to be suitable spawning habitat (Fig. 7). The actual number of habitat cells that were suitable for spawning at Wooded Island was zero; thus, none of the sites predicted to have redds had redds. The predicted spawning sites at Wooded Island contained similar physical features as found within spawning areas at Locke Island, i.e., mean lateral slope 3%, water depth 2.9 m, and velocity 1.7 $m \cdot s^{-1}$.

Discussion

Spatial pattern analysis confirmed that fall chinook salmon redds at the Locke Island site occurred in clusters. Even though the number of redds varied almost twofold in 1994 and 1995, the locations of these clusters were similar. In both years, the majority of redds occurred in clusters and were within 30 m of each other. In 1994 when redd densities

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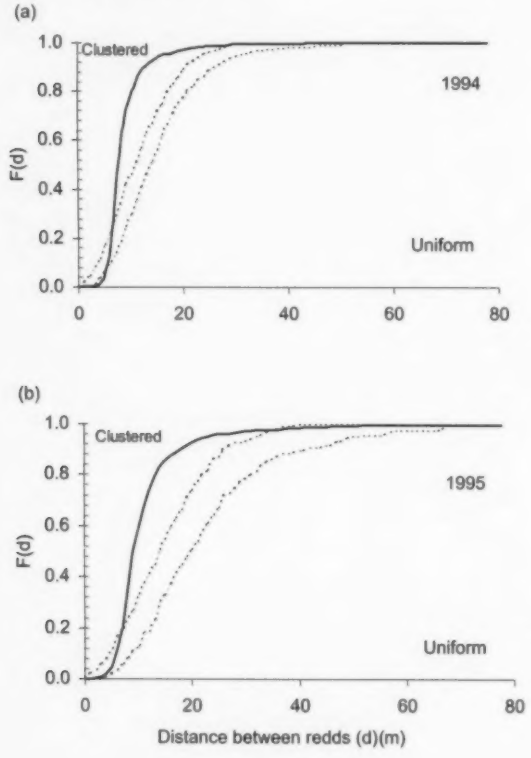
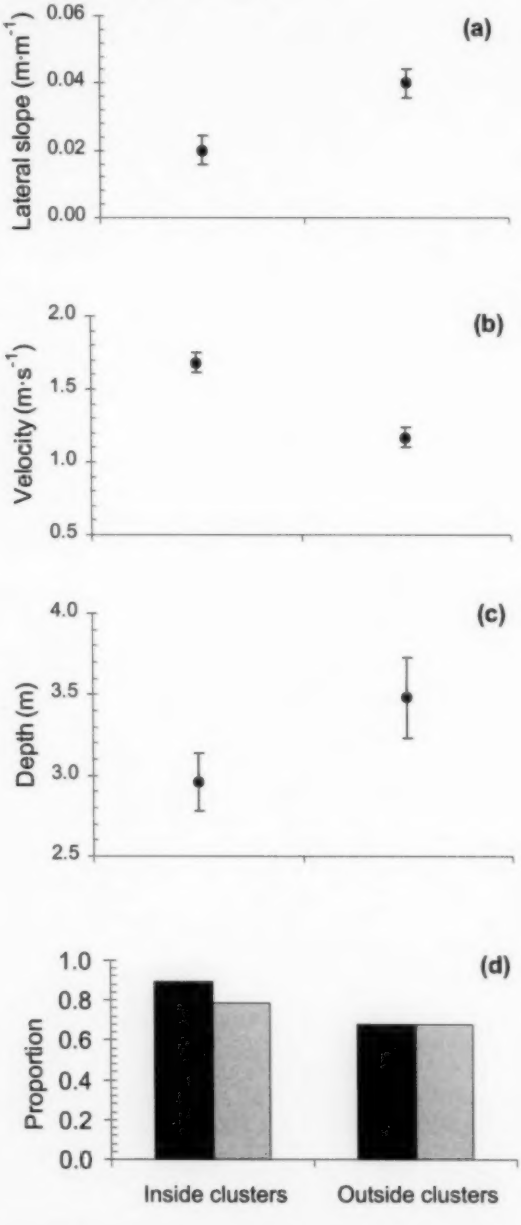
Fig. 5. Refined nearest-neighbor analysis of the spatial point pattern of fall chinook salmon redds at Locke Island. The $F(d)$ is the cumulative distribution function that describes the probability that the nearest neighbor to a redd is with a given distance d; the formula is given in the text. The $F(d)$ is presented for redds in (*a*) 1994 and (*b*) 1995. The solid lines for $F(d)$ represent a cumulative histogram of the nearest-neighbor distances calculated from the data. The broken lines represent the upper and lower boundaries of the 95% confidence envelope of 100 Monte Carlo simulations. Deviation of the empirical distributions (solid lines) above or below the confidence envelope indicates redd distributions that are clustered or uniform, respectively. Random redd distribution is indicated when the solid line is contained within the confidence envelope.



were higher, there was strong evidence of a uniform distribution of redds within the clusters at distances of 2–5 m. The crossover distance (i.e., 2–5 m) where patterns switched from uniform to clustered was approximately the distance between the centers of two adjacent redds. In other words, at the spatial scale of a spawning area, redds exhibited high fidelity and occurred in clusters, but within each cluster, redds were uniformly spaced.

Uniformity in the spatial patterns of redds within clusters at Locke Island in 1994 likely resulted from interactions between individuals competing for space (Ludwig and Reynolds 1988). Interactions between neighboring chinook salmon females influenced redd site selection within a cluster of redds in the Nechako River, British Columbia (Neilson and Banford 1983). That the redd densities within clusters were high in 1994 suggests that the available space was

Fig. 6. Habitat measured inside and outside fall chinook salmon redd clusters within the Locke Island study site during 1994: (*a*) lateral slope, (*b*) water column velocity, (*c*) water depth, and (*d*) the proportion of all substrate samples where the dominant substrate (solid bars) was in the size range of 5.1–15.1 cm and where the subdominant substrate (hatched bars) was in the size range of 2.5–7.6 cm. Bars above and below the points represent the 95% confidence interval of the means.



exhaustively partitioned among redds. Competition for spawning locations within clusters of redds apparently occurred in the Locke Island study site despite the fact that escapements of fall chinook salmon to the Hanford Reach in

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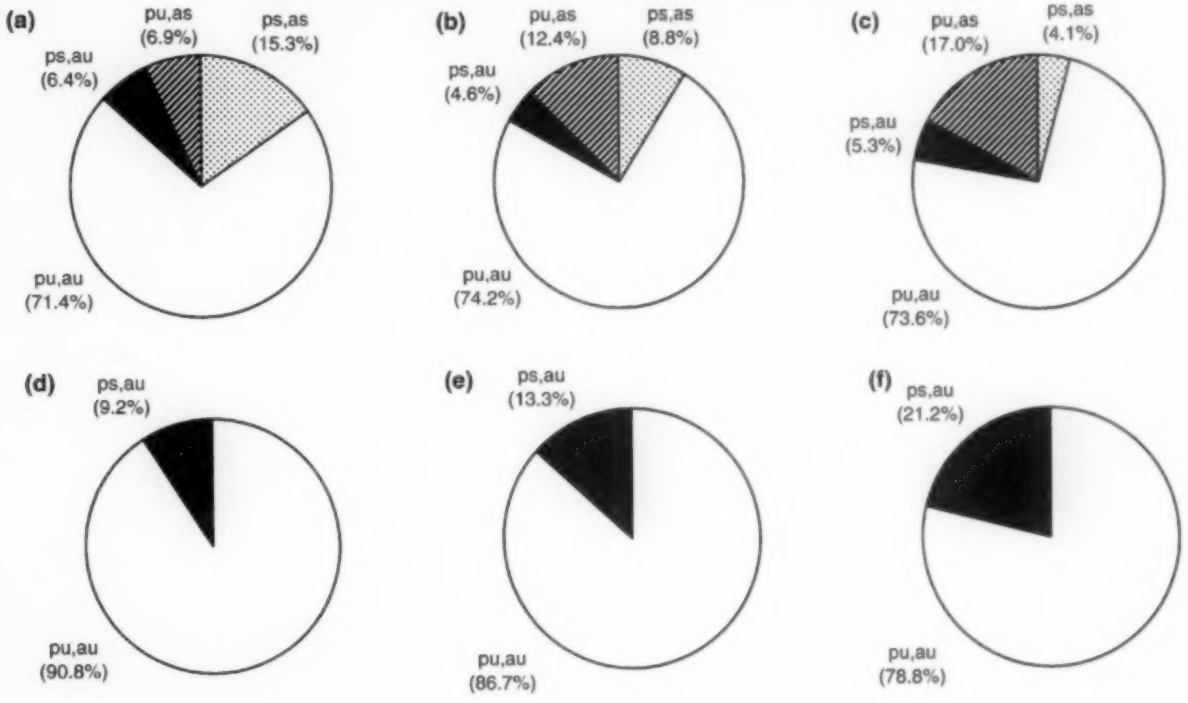
Table 1. Stepwise model selection results from multiple logistic regression of hydraulic simulation data explaining the distribution of fall chinook salmon spawning at Locke Island in 1994.

Variable	Estimated regression coefficient	Wald χ^2	P	95% CI for odds ratios	
				Lower	Upper
Lateral slope	−27.1±8.47	10.2	0.0014	0.65	0.90
Q_{ave} depth	−1.8±0.27	44.7	<0.0001	0.32	0.54
Q_{ave} velocity	31.0±6.69	21.5	<0.0001	5.98	82.29
Q_{ave} velocity^2	−8.6±2.17	15.9	<0.0001	0.28	0.64
Intercept	−20.9±5.0				

Note: Regression statistics for the spawning habitat model (4 df) were log likelihood = 200.73 (intercept only) and 101.97 (intercept and covariates). Regression coefficients are depicted as estimate ± 1 SE. The significance level for stepwise selection was 0.05. Q_{ave} depth, Q_{ave} velocity, and Q_{ave} velocity^2 are the simulated depths and velocities at the 12-h average discharge (2034 $m^3 \cdot s^{-1}$).

Fig. 7. A logistic regression model was used to predict fall chinook salmon spawning habitat suitability in 1995 at Locke Island and Wooded Island at Q_{min}, Q_{ave}, and Q_{max} hourly discharges. (*a*) Locke Island at Q_{min} (1896 $m^3 \cdot s^{-1}$); (*b*) Locke Island at Q_{ave} (2474 $m^3 \cdot s^{-1}$); (*c*) Locke Island at Q_{max} (3481 $m^3 \cdot s^{-1}$); (*d*) Wooded Island at Q_{min} (2285 $m^3 \cdot s^{-1}$); (*e*) Wooded Island at Q_{ave} (2974 $m^3 \cdot s^{-1}$); (*f*) Wooded Island at Q_{max} (3 420 $m^3 \cdot s^{-1}$). p, predicted; a, actual; s, spawning habitat suitable; u, spawning habitat unsuitable.



1994 were only 55% of peak escapement observed in 1987 (Dauble and Watson 1997).

If sites suitable for spawning were patchily distributed, as the nonrandom distribution of redds suggested, they could have a unique set of attributes that were not found throughout the entire study area. An alternative interpretation might be that spawning salmon do not have strong habitat-specific fidelity but choose to spawn adjacent to sites chosen by the earliest arriving individuals in a season (Duker 1981). For example, brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) were found to selectively spawn in areas of the streambed that had been disturbed by earlier spawners (Essington et al. 1998). However, the high rates of reoccupation of redd clusters between years suggest that cluster location is not random and support the site-specific fidelity interpretation. Specific habitat utilization and clustering of salmon spawning has been observed in other populations. For example, chinook salmon in the Kamchatka River, Russia, spawned in distinct patches, even though apparently similar habitat was available nearby (Vronskiy 1972). Chapman (1943) noted concentrated chinook salmon spawning in the mainstem Columbia River below Kettle Falls, Washington, where "the greater part of the river was not in use." Clusters of chinook salmon spawning in the

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Nechako River corresponded to river bottom topography and substrate, with fish preferentially spawning along the lateral gravel ridges (Neilson and Banford 1983).

PHABSIM estimates spawning habitat using criteria based on published or measured redd selection data (Bovee 1995). The use of river-specific suitability criteria is currently believed to be the best method for predicting spawning habitat for salmonids within rivers and streams (Shirvell 1989; Bovee 1995). Our logistic regression model used characteristics of spawning habitat measured within a local spawning area and represented the suitability criteria over a finer spatial scale than river-specific criteria. The analysis suggested that 9–22% of the riverbed at Locke Island and 9–21% of the riverbed at Wooded Island were suitable as spawning habitat for fall chinook salmon. These estimates were closer to the actual amount (~20% at Locke Island and 0% at Wooded Island) than the results from the previous study where river-specific criteria were used (estimates of 50%; Geist et al. 1997). Better predictions were possible because our approach removed much of the variation between spawning areas that are widely separated and resulted in a narrower range of suitable spawning habitat. That the logistic regression analysis, based on spawning habitat characteristics measured at Locke Island, predicted higher spawning utilization than actually occurred at Wooded Island showed that chinook salmon did not respond to habitat in a similar manner at each site. Additional evaluations of the logistic regression model at other high-use spawning areas are needed and may show that each individual spawning area contains a unique combination of physical habitat.

The aerial photographs from Locke Island in 1994 and 1995 did not show fall chinook salmon redds above the 1415 $m^3 \cdot s^{-1}$ flow elevation. The minimum hourly discharges in 1994 (1453 $m^3 \cdot s^{-1}$) and 1995 (1896 $m^3 \cdot s^{-1}$) were above this level, suggesting that redd clustering was not a function of minimum flows. It would appear from our analysis in 1994 when the minimum flows were near the 1415 $m^3 \cdot s^{-1}$ flow level that the clustering of redds may have been in response to the rapid change in discharge because fish appeared to select habitat at the highest flow elevation that did not become dewatered in a 24-h period. However, the results in 1995 do not support this, as the minimum flows were substantially higher than in 1994 but the location of spawning was very similar. This would suggest that factors other than flow fluctuations were responsible for redd clustering. That the logistic regression analysis showed that redd locations were more correlated with velocity and depth simulated at the average flow conditions than at the minimum or maximum flows suggests that fish were responding to flow conditions that occurred over a longer time period than represented by minimum or maximum hourly values.

Approximately 29–56% of the sites at Locke Island and all the sites at Wooded Island predicted to have redds using the physical attributes did not have redds (i.e., errors of commission). Some of this error can be explained by the lower number of redds in 1995. The logistic regression model was constructed using redd data from 1994, so not all the sites predicted to be suitable in 1995 could be filled. However, even in 1994 at Locke Island when redd densities were near capacity (D.R. Geist, unpublished data), errors of commission resulted. Most of these errors occurred adjacent to large clusters of redds. This suggested that although the depth, lateral slope, and velocity in these locations were similar to those in the clusters, some other key attribute was different and resulted in fewer fish colonizing these areas. In contrast, some areas of streambed predicted to be unsuitable by our model had redds (i.e., errors of omission). Errors of omission may result from spawners being forced into "suboptimal" habitat located on the fringes of the clusters (Neilson and Banford 1983). Most of these errors occurred near the boundaries of the clusters where the lateral slope increased due to a change in riverbed form.

In either case, there was some unmeasured factor(s) that influenced redd site selection. For example, the long, sinuous, and narrow gaps frequently observed within the redd clusters in the Hanford Reach suggest that spawning is controlled by geomorphic features of the river bottom (i.e., sedimentary structures). The shapes of redd clusters were very similar to those commonly observed in longitudinal bars and channels deposited by gravelly braided rivers (e.g., Rust and Koster 1984). Hydraulic processes that form the longitudinal bars and channels in the river directly influence the topography (depth and slope) and the sediment size exposed on the river bottom. Geist and Dauble (1998) proposed that geomorphic features promote groundwater – surface water interactions within hyporheic habitats and may play a role in spawning site selection by fall chinook salmon. In fact, upwelling from hyporheic habitats into the river in Locke Island spawning areas was greater than upwelling into nonspawning areas at Locke Island and Wooded Island (Geist 2000). Further, the upwelling in spawning areas contained more oxygen and was composed of a higher proportion of river water than upwelling in nonspawning areas. These upwelling characteristics could provide cues that adult fall chinook salmon used to locate preferred spawning habitat.

Fisheries managers use hydraulic models like PHABSIM because they provide a conservative estimate of fish habitat. When river-specific habitat suitability criteria are used, this modeling approach may not help define the relative importance of physical habitat features that control spawner use at the cluster scale. The risk in overestimating suitable spawning habitat is that many recovery goals for anadromous salmonids are not supported by empirical data. Evaluating habitat characteristics at the scale of redd clusters provides an alternative means for describing specific physical features that influence salmon spawning and improves the overall understanding of factors affecting redd site selection. We believe that this will lead to more realistic estimates of spawning habitat capacity, which will result in better recovery goals and a more efficient use of recovery resources.

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Hyporheic discharge of river water into fall chinook salmon (*Oncorhynchus tshawytscha*) spawning areas in the Hanford Reach, Columbia River

David R. Geist

Abstract: Fall chinook salmon (*Oncorhynchus tshawytscha*) spawned predominantly in areas of the Hanford Reach of the Columbia River where hyporheic water discharged into the river channel. This upwelling water had a dissolved solids content (i.e., specific conductance) indicative of river water and was presumed to have entered highly permeable riverbed substrate at locations upstream of the spawning areas. Hyporheic discharge zones composed of undiluted ground water or areas with little or no upwelling were not used by spawning salmon. Rates of upwelling into spawning areas averaged 1200 $L \cdot m^{-2} \cdot day^{-1}$ (95% CI = 784–1665 $L \cdot m^{-2} \cdot day^{-1}$) as compared with approximately 500 $L \cdot m^{-2} \cdot day^{-1}$ (95% CI = 303–1159 $L \cdot m^{-2} \cdot day^{-1}$) in nonspawning areas. Dissolved oxygen content of the hyporheic discharge near salmon spawning areas was about 9 $mg \cdot L^{-1}$ (±0.4 $mg \cdot L^{-1}$) whereas in nonspawning areas, dissolved oxygen values were 7 $mg \cdot L^{-1}$ (±0.9 $mg \cdot L^{-1}$) or lower. In both cases, dissolved oxygen of the river water was higher (11.3 ± 0.3 $mg \cdot L^{-1}$). Physical and chemical gradients between the hyporheic zone and the river may provide cues for adult salmon to locate suitable spawning areas. This information will help fisheries managers to describe the suitability of salmon spawning habitat in large rivers.

Résumé : Le saumon quinnat (*Oncorhynchus tshawytscha*) d'automne fraye principalement dans des secteurs du tronçon Hanford du Columbia où des eaux hyporhéiques entrent dans le chenal. Ces eaux remontantes ont une teneur en solides dissous (ou conductance spécifique) caractéristique des eaux fluviales et on pense qu'elles pénètrent un substrat très perméable en amont des frayères. Les saumons étudiés n'ont pas frayé dans les secteurs où entrent des eaux souterraines hyporhéiques non diluées ni dans les secteurs où la remontée d'eau est faible ou nulle. Les taux de remontée d'eau dans les frayères étaient en moyenne de 1200 $L \cdot m^{-2} \cdot jour^{-1}$ (intervalle de confiance de 95% = 784 à 1 665 $L \cdot m^{-2} \cdot jour^{-1}$), comparativement à 500 $L \cdot m^{-2} \cdot jour^{-1}$ (intervalle de confiance de 95% = 303 à 1159 $L \cdot m^{-2} \cdot jour^{-1}$) ailleurs. La teneur en oxygène dissous dans les eaux hyporhéiques qui apparaissent près des frayères était d'environ 9 $mg \cdot L^{-1}$ (±0,4 $mg \cdot L^{-1}$) tandis qu'ailleurs elle était de 7 $mg \cdot L^{-1}$ (±0,9 $mg \cdot L^{-1}$) ou moins. La teneur en oxygène dissous des eaux du fleuve (11,3 ± 0,3 $mg \cdot L^{-1}$) était supérieure à ces deux valeurs. Les gradients physiques et chimiques entre la zone hyporhéique et l'eau du fleuve peuvent orienter les saumons adultes dans la recherche de bonnes frayères. Ces données devraient aider les gestionnaires à repérer les frayères propices au saumon dans les grands cours d'eau.

[Traduit par la Rédaction]

Introduction

Fall chinook salmon (*Oncorhynchus tshawytscha*) redds are often clustered, providing evidence that spawning areas contain specific habitat characteristics that are not widely available (Chapman 1943; Geist and Dauble 1998; Geist et al. 2000). Lateral slope of the river bottom and mean water column velocity are significantly different inside and outside fall chinook salmon redd clusters in the Hanford Reach of the Columbia River (Geist et al. 2000). However, approximately 30–60% of the areas predicted to have redds based on channel features did not, which suggested that discharge from hyporheic habitats may influence redd site selection.

There is circumstantial evidence that hyporheic discharge influences redd site selection of fall chinook salmon (reviewed in Geist and Dauble 1998); however, no quantitative studies had been conducted in large rivers. Understanding the interaction of ground water and surface water within hyporheic habitats of large rivers will allow fisheries managers to better define fall chinook salmon spawning habitat requirements. This information is needed because restoration efforts that are presently being planned include alteration of mainstem habitats through dam removal and reservoir drawdown and reintroduction of fall chinook salmon into mainstem habitats that have been blocked by hydroelectric development (Northwest Power Planning Council 1994; Independent Scientific Group 1996).

The purpose of this study was to evaluate the relationship between hyporheic discharge and fall chinook salmon spawning site selection in the Hanford Reach, an alluvial floodplain section of the Columbia River. In this paper, hyporheic discharge includes a mix of phreatic ground water and river water that discharges from the hyporheic zone into the river channel (e.g., Vervier et al. 1992; Harvey and Bencala 1993;

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D.R. Geist. Ecology Group, Pacific Northwest National Laboratory, P.O. Box 999, MS K6-85, Richland, WA 99352, U.S.A. email: david.geist@pnl.gov

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Brunke and Gonser 1997). Phreatic ground water originates beneath land areas and contains a significant component of dissolved solutes derived from a long residence time in the subsurface (Freeze and Cherry 1979). Hyporheic discharge was assumed to affect spawning site selection by providing cues (chemical, temperature, and physical) for prespawning adults to locate spawning reaches (usually 2–5 km in length). Once these reaches were "discovered," hyporheic discharge was assumed to correlate with the distribution of redd clusters (500–800 m in length, 120 m in width; Geist et al. 2000) within these river reaches. I hypothesized that the physical and chemical characteristics of the hyporheic discharge in areas where fall chinook salmon spawned (both at the reach and cluster scale) would be different from the characteristics of the discharge in nonspawning areas. This hypothesis was tested over a range of river conditions and spawning seasons (1995–1997) between and within two sites previously studied for fall chinook salmon spawning habitat suitability (Geist et al. 2000).

Methods

Study area

The Hanford Reach is the last unimpounded section of the mainstem Columbia River in the United States (Geist et al. 2000). The association between fall chinook salmon spawning and hyporheic discharge was examined at Locke Island (river kilometres 596-600) and Wooded Island (river kilometres 560–563) (Geist et al. 2000). The Locke Island study site has much higher fall chinook salmon redd densities than the Wooded Island site. For example, the average annual fall chinook salmon redd count at Locke Island from 1990 to 1995 was 513 (range 340–855), while the average redd count at Wooded Island during the same time period was 10 (range 0–32) (D. Dauble, Pacific Northwest National Laboratory, Richland, Wash., unpublished data). Because of rapid water releases during periods requiring peak power production at an upstream dam (i.e., Priest Rapids Dam), water levels within the study sites commonly fluctuate up to 2 $m \cdot day^{-1}$. Details about these study sites and site-specific fall chinook salmon spawning habitat use are described in Geist et al. (2000).

Piezometer installation and monitoring

During the fall chinook salmon spawning seasons (October and November; Dauble and Watson 1997) from 1995 to 1997, minipiezometers and internal-drive-rod piezometers were installed within the study sites. Minipiezometers were installed following the methods described in Lee and Cherry (1978). Each minipiezometer consisted of a 10-cm piece of polyethylene tube (9.5 mm outside diameter, 6.4 mm inside diameter) that was attached on one end to an expendable drive point and on the other end to a section of polyethylene tubing (6.4 mm outside diameter, 4.8 mm inside diameter). The length of the tubing (6.4 mm in diameter) that was attached to the screened tubing (9.5 mm in diameter) was dependent on installation depth below the riverbed and the depth of the river. There were approximately twelve 3.2-mm-diameter perforations in each 10-cm section of tube. The perforated section of the tube was wrapped three times with 240-μm nitex screen and secured with adhesive. Internal-drive-rod piezometers were installed following the methods described in Geist et al. (1998). Each internal-drive-rod piezometer was constructed of a 184- or 215-cm section of steel pipe (4.2 cm outside diameter, 3.5 cm inside diameter) with a solid steel drive point welded to one end and a variable-length extension attached to the other end. The lower 30-cm section of the piezometer was perforated with 24 holes (3.2 mm in diameter) that were sometimes screened using a 2.5-cm-diameter line strainer screen. Screens were used in locations that contained a high amount of fine sediment. Screens did not affect piezometer performance or the hyporheic measurements.

Piezometers were installed within the river channel in clusters of three or four, with each piezometer installed at a different depth below the riverbed. The distance between piezometers within a piezometer cluster usually did not exceed 30 cm. I attempted to locate piezometers at depths between 30 and 150 cm below the riverbed. This range was based on the depth of an average fall chinook salmon redd (~45 cm) and the practical limits of the installation technique. The average installation depth (i.e., below the riverbed) of piezometers in 1995, 1996, and 1997 was 97, 71, and 99 cm, respectively. The water depth of the river where piezometers were installed was usually ≤1 m, although three piezometers were installed at Locke Island in water that was 3–4 m deep. These three piezometers were sampled with the use of polyethylene tubes (6.4 mm outside diameter, 4.8 mm inside diameter) that were attached to the tops of the piezometers at the bed of the river and extended to the shoreline (~300 m).

Piezometer clusters were placed in each of the study sites in relation to the location of fall chinook salmon spawning. All piezometers placed at Wooded Island were assumed to be representative of nonspawning habitat because few fall chinook salmon spawn there. Piezometers at Locke Island were placed within and adjacent to fall chinook salmon spawning areas as well as within areas that physical habitat models incorrectly predicted spawning to occur (Geist et al. 2000) (shaded area in Fig. 1). Spatial pattern analysis of redd locations depicted through aerial photography in 1994 and 1995 determined that clusters of redds at Locke Island usually contained more than 20 redds (Geist et al. 2000). Therefore, a geographic information system was used to calculate the average distance from each piezometer cluster to the nearest 20 fall chinook salmon redds. The average distance from piezometer clusters to the nearest 20 redds was 48 m (range 26–68 m) and 128 m (range 93–169 m) for "spawning" and "nonspawning" piezometers, respectively, at Locke Island. The resulting number of piezometers installed at both sites is shown in Table 1.

Once in place and operational, the piezometers were used to sample specific conductance (microsiemens per centimetre at 25°C), water temperature (degrees Celsius), dissolved oxygen (DO, milligrams per litre), and hydraulic head (h, centimetres) of the hyporheic water. These parameters were also measured on a river sample taken contiguous to the piezometer. Specific conductance was the primary measure used to differentiate phreatic ground water from surface water; specific conductance of undiluted ground water adjacent to the Hanford Reach averages 300–400 $\mu S \cdot cm^{-1}$, while that of the river water averages approximately150 $\mu S \cdot cm^{-1}$ (Peterson and Johnson 1992; Dresel et al. 1995). The differences in temperature and hydraulic head (Δh) were based on the piezometer reading (hyporheic water) minus the reading from its contiguous river sample. Individual piezometers were sampled an average of seven, four, and two times during the spawning seasons in 1995, 1996, and 1997, respectively. Measurements of water temperature and specific conductance were made on a water sample that was either extracted from the piezometer using a low-volume peristaltic pump (the volume pumped for analysis was at least equal to the storage volume of the piezometer) or sampled in situ within the piezometer after pumping. DO was measured in a sample that was continuously pumped from the piezometer; placing the DO probe directly in the piezometer to obtain DO measurements did not prove satisfactory. Permeability of sediments was large relative to pumping rate so that the measured water was not aerated. All river measurements were made in situ immediately adjacent to the piezometer. Measurements of specific conductance and temperature were made with a temperature/conductivity meter (YSI model 30) and DO was sampled with a DO meter (YSI model 95) and (or) with the use of a portable HACH kit (model DR2000).

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Fig. 1. Locke Island study site where comparisons of hyporheic discharge were conducted between fall chinook salmon spawning and nonspawning areas. Clusters of piezometers installed in 1996 (squares) and 1997 (triangles) were used to collect this information. Open symbols show piezometer clusters that were representative of spawning areas, whereas closed symbols show piezometer clusters representative of nonspawning areas (see text for definitions of spawning and nonspawning areas). Dots represent fall chinook salmon redds as digitized from aerial photographs taken in 1995. The shaded oval represents an area of river bottom where a previous study (Geist et al. 2000) predicted that spawning should occur but did not. Labeled piezometers (L20, LDW2, and L41) are highlighted to show individual piezometers where data loggers were installed in 1996 to monitor the change in vertical potentials under periods of fluctuating river discharge.

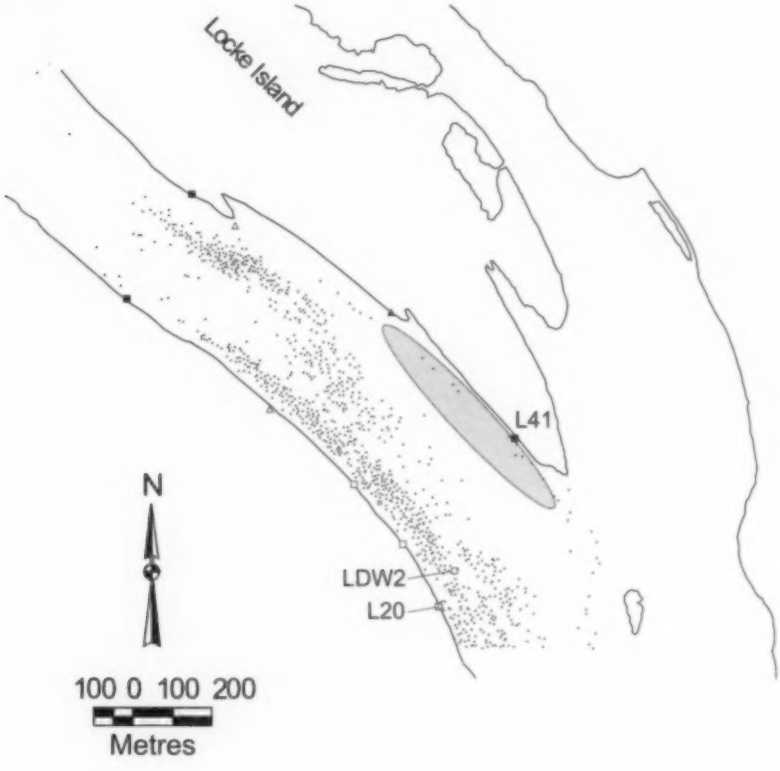

Table 1. Number of individual piezometers (piezometer groups in parentheses) installed at Locke Island and Wooded Island during the fall chinook salmon spawning seasons from 1995 through 1997.

Year	Wooded Island	Locke Island	
		Spawning areas	Nonspawning areas
1995	12 (4)	8 (3)	9 (5)
1996	9 (4)	12 (5)	11 (3)
1997	9 (3)	14 (3)	5 (2)

Note: The definition of spawning areas is provided in the text.

Hydraulic head measurements were taken from the top of the piezometer using an electrical interface measuring tape (Solinst). The hydraulic head measurements were used to calculate the vertical hydraulic gradient (VHG) for each piezometer:

$$\mathrm{VHG} = \frac{\Delta h}{L}$$

where Δh was the hydraulic head inside the piezometer minus the hydraulic head of the river (centimetres) and L (centimetres) was the distance below the riverbed to the top of the piezometer perforations. The VHG is a unitless index with positive values indicative of an energy gradient sufficient to produce upwelling (i.e., hyporheic discharge zones) and negative values indicative of a gradient sufficient to produce downwelling (i.e., hyporheic recharge zones) (Freeze and Cherry 1979; Dahm and Valett 1996).

Hydraulic conductivity of the riverbed sediments was measured at each study site in 1997 using a modified slug test (Freeze and Cherry 1979; Dahm and Valett 1996). Slug tests were performed inside of internal-drive-rod piezometers (Geist et al. 1998) that were installed at depths ranging from 40 to 152 cm below the riverbed. Each test consisted of inserting a slug rod (2.5 cm outside diameter) into the piezometer and allowing the hydraulic head to stabilize, measuring the initial hydraulic head within the piezometer, and then rapidly withdrawing the slug rod and recording the time for the head to recover. Hydraulic head was measured during the test every 0.25 s with a pressure transducer (KPSI model 30S-112-0010) and data logger (Campbell Scientific CR10). Two replications were conducted at each piezometer; 10 piezometers were measured at Locke Island and six at Wooded Island. Hydraulic conductivity (K, centimetres per second) was calculated with the following formula (Hvorslev 1951):

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$$K = \frac{r^2 \ln(L/R)}{2LT_0}$$

where r is the radius of piezometer (1.75 cm), R is the radius of the piezometer screen (1.27 cm), L is the length of piezometer screen (30 cm), and T_0 is the time for the hydraulic head in the piezometer to recover to 37% of initial change after the slug rod is removed. An estimate of the specific discharge (v, centimetres per second) into the Columbia River was made using the Darcy relationship (Freeze and Cherry 1979):

$$v = K \times \mathrm{VHG}$$

where K and VHG were as previously defined.

The effect of diurnal fluctuations of river stage (i.e., discharge) on hyporheic discharge was monitored in five piezometer groups that were representative of the study site conditions. A paired set of piezometer groups was monitored at nonspawning areas at Wooded and Locke islands. A second comparison was done between three piezometer groups installed across the channel within spawning and nonspawning areas at the Locke Island site. Absolute pressure (i.e., hydrostatic plus barometric pressure) of hyporheic and surface waters was recorded simultaneously every 15 min using self-contained data loggers (Levelogger model 3001, Solinst) that were placed within internal-drive-rod piezometers and river standpipes. Barometric pressure was recorded simultaneously using a pressure transducer (Vaisala model PTB101B) connected to a data logger (Campbell Scientific CR10) located along the bank of the river. The hydraulic head of the hyporheic and surface water was determined by subtracting the barometric pressure from the absolute pressure readings. The VHG was calculated from these readings.

Data analysis

Physicochemical data and hydraulic data were plotted and inspected for normality and equal variances. Differences in physicochemical data of the hyporheic and surface waters between the Locke Island and Wooded Island sites were tested statistically with analysis of variance and regression ($\alpha = 0.05$). With the exception of the hydraulic conductivity and DO data that were collected in 1997, the between-site comparisons were performed only on data collected in 1995. This was because data for the other parameters from Wooded Island were not taken in 1996 and 1997. The paired piezometer clusters where continuous data loggers were installed were used to compare the effect of fluctuating river discharge on the vertical potential between hyporheic and surface waters. The effects of piezometer depth and river stage (water surface elevation of river) on physicochemical parameters were evaluated at each site using regression analysis. Water surface elevations were acquired from continuous river stage recorders located throughout the Hanford Reach and operated by Bechtel Hanford, Inc. (Richland, Wash.)

Comparisons of the physicochemical data collected in fall chinook salmon spawning and nonspawning areas within the Locke Island site were also done using analysis of variance and regression analysis. The within-site comparison was conducted on a 1200 × 400 m area within the Locke Island site (Fig. 1) that was previously evaluated for spawning habitat suitability (Geist et al. 2000). A logistic regression model that was based on lateral slope of the riverbed, water velocity, and water depth incorrectly predicted spawning to occur within this area (shaded area in Fig. 1). The physicochemical data used in the comparisons included specific conductance and temperature (1996 and 1997), DO (1997), and hydraulic head and VHG (1996). The data from the continuous water level recordings were used to evaluate differences in response of hyporheic waters to fluctuating river discharge in spawning and nonspawning areas (Fig. 1). The effects of piezometer depth and river stage on physicochemical parameters of spawning and nonspawning areas were also evaluated using regression analysis.

Results

From 1995 through 1997, the daily average discharge of the river at Priest Rapids Dam during the fall chinook salmon spawning season (October and November) was similar and averaged 3154 $m^3 \cdot s^{-1}$ (range 2875 $m^3 \cdot s^{-1}$ in 1995 to 3453 $m^3 \cdot s^{-1}$ in 1997). The distribution of chinook salmon spawning at Wooded and Locke islands was consistent with that of previous years. In 1995, 1996, and 1997, there were three, five, and 28 fall chinook salmon redds counted at Wooded Island and 340, 392, and 607 redds counted at Locke Island (D. Dauble, Pacific Northwest National Laboratory, Richland, Wash., unpublished data).

Between-site comparisons

The positive VHG and hydraulic head difference (Δh) between hyporheic and surface waters indicated that hyporheic water was predominantly upwelling into the river at the Locke Island and Wooded Island sites. Average VHG and Δh values at Locke Island were significantly higher than values at Wooded Island ($P = 0.002$ and $P = 0.0001$, respectively) (Table 2). In addition, hyporheic discharge into the river at Wooded Island contained a significantly greater proportion of ground water (i.e., higher specific conductance) than the upwelling into the river at Locke Island ($P = 0.005$) (Table 2).

Specific discharge calculations gave an average flux out of the sediments on the order of 9.0×10^{-4} $cm \cdot s^{-1}$ at Locke Island and 3.0×10^{-4} $cm \cdot s^{-1}$ at Wooded Island. However, there was no difference between sites in the temperature gradient between hyporheic and surface waters ($P = 0.41$) (Table 2) or in the DO concentrations in the hyporheic waters ($P = 0.21$) (Table 2). Hydraulic conductivity of the riverbed sediments also did not differ between sites ($P = 0.42$), averaging 0.02 (SD = 0.01) and 0.03 (SD = 0.03) $cm \cdot s^{-1}$ at Locke Island and Wooded Island, respectively.

River stage and physicochemical characteristics were weakly or not correlated (i.e., $r^2 \leq 0.10$) at both sites. This was also true when VHG was monitored over a range of river discharges that are typical in a 24-h period during the spawning season (Fig. 2). At Wooded Island, river stage changed approximately 1.2 m in a 16-h period, but VHG remained approximately constant within a group of piezometers (Fig. 2*a*). A 0.7-m change in river stage within nonspawning piezometers at Locke Island over a 24-h period also produced little change in VHG (Fig. 2*b*).

The physicochemical characteristics at both sites did not change with increasing distance below the riverbed, as indicated by the nonsignificant correlation between piezometer depth and all the measured parameters except specific conductance at the Locke Island site (all $P > 0.05$). However, the hydraulic conductivity tests showed that the time to recovery of the hydraulic head within the hyporheic waters at Wooded Island increased significantly with increasing piezometer depth ($P = 0.02$, $r^2 = 0.78$) (Fig. 3). At Locke Island, recovery was independent of depth ($P = 0.42$, $r^2 = 0.08$). These results suggested that substrate permeability decreased with increasing depth at Wooded Island but not at Locke Island.

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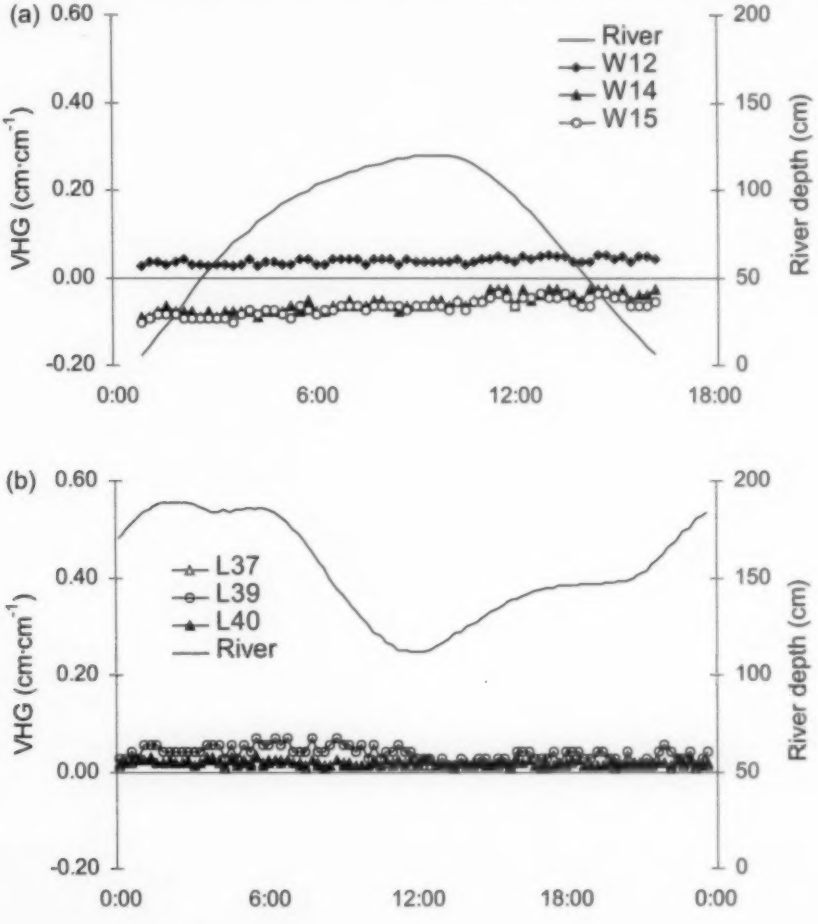
Table 2. Average values (±1 SD in parentheses) of physicochemical data collected from the river and piezometers installed and monitored at the Locke Island and Wooded Island study sites during the spawning season (October and November) in 1995 and 1997.

Parameter	Wooded Island	Locke Island	River at both sites
VHG (1995)	0.01 (0.04)	0.05 (0.08)	na
Δh (hyporheic – river, cm) (1995)	0.65 (2.9)	4.96 (7.4)	na
Specific conductance ($\mu S \cdot cm^{-1}$) (1995)	188.6 (43.4)	161.3 (62.7)	133.5 (3.9)
ΔT (hyporheic – river,°C) (1995)	–0.04 (0.7)	0.08 (0.9)	na
DO ($mg \cdot L^{-1}$) (1997)	7.3 (0.8)	7.7 (1.3)	11.5 (0.2)

Note: na, not applicable.

Fig. 2. Continuous measurements of VHG between hyporheic and surface waters at (*a*) Wooded Island (October 23, 1997) and (*b*) Locke Island (December 13, 1996). River depth represents the depth of the river within the standpipe attached to the piezometer group and is a surrogate for river discharge. The daily average discharge at Priest Rapids Dam was 3256 and 2966 $m^3 \cdot s^{-1}$ on October 23, 1997, and December 13, 1996, respectively. The time scale (*x*-axis) in Fig. 2*a* is 15.5 h and in Fig. *b* is 24 h.



Within-site comparisons

Positive Δh and VHG values indicated that hyporheic water upwelled into the river in both spawning and nonspawning areas within the Locke Island study site (Figs. 4*a* and 4*b*), but Δh and VHG were significantly higher in spawning areas than in nonspawning areas ($P = 0.0005$ and $P = 0.002$, respectively). Assuming a hydraulic conductivity of 0.02 cm·s–1 in both spawning and nonspawning areas, there was an upward flux out of the sediments of approximately 1.4×10^{-3} $cm \cdot s^{-1}$ ($1200\ L \cdot m^{-2} \cdot day^{-1}$) in spawning areas and 6.0×10^{-4} $cm \cdot s^{-1}$ ($518\ L \cdot m^{-2} \cdot day^{-1}$) in nonspawning areas.

Measurements of specific conductance showed that the hyporheic discharge in nonspawning areas contained significantly more phreatic ground water than the discharge in

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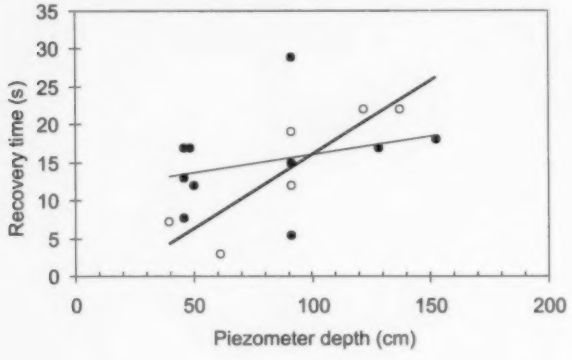
Fig. 3. Recovery time to 37% of the initial hydraulic head following a slug test within piezometers installed at Locke Island and Wooded Island. Solid circles and the thin line represent the empirical data and trend line, respectively, for the Locke Island site. Open circles and the thick line represent the empirical data and trend line, respectively, for the Wooded Island site. The trend line at the Wooded Island site was significant ($P = 0.02$, $r^2 = 0.78$) but was not significant at Locke Island ($P = 0.42$, $r^2 = 0.08$).



spawning areas ($P < 0.0001$, 1996 and 1997) (Fig. 4*c*). The specific conductance of the hyporheic discharge increased approximately 4 $\mu S \cdot cm^{-1}$ ($P < 0.0001$, $r^2 = 0.72$) with every 10-m increase in distance away from redd clusters. Hyporheic discharge within spawning areas was more oxygenated than discharge in nonspawning areas ($P = 0.02$), with average DO in spawning and nonspawning areas measured at 8.8 (SD = 0.4) and 6.7 (SD = 0.9) $mg \cdot L^{-1}$, respectively. There was no difference in the temperature gradient between hyporheic and surface waters in spawning and nonspawning areas in 1996 ($P = 0.44$) and 1997 ($P = 0.48$) (Fig. 4*d*).

River stage was not strongly related to the physicochemical characteristics of the hyporheic waters when measurements were taken at single points in time. In contrast, continuous measurement of hydraulic head in the hyporheic and surface waters showed a relationship between VHG and river stage in the spawning area but not in the nonspawning area (Fig. 5). In the nonspawning area, VHG between hyporheic and surface waters approximately 1 m below the riverbed (L41) was not highly responsive to the fluctuating river discharge (Fig. 5*a*). The response in the spawning areas was different depending on piezometer location and depth. In both areas, the VHG was related to river stage. In the nearshore piezometer (L20 on Figs. 1 and 5*b*), the response of the hydraulic head of the hyporheic water 72 cm below the bed was almost instantaneous and actually switched from positive to negative as the river level increased. In contrast, the VHG between the hyporheic and surface waters 61 cm below the bed and the river in the offshore piezometer (LDW2 on Figs. 1 and 5*b*) always remained positive and responded much more slowly than the nearshore piezometer (L2); the time delay between maximum river depth and minimum VHG was 10–15 h, while the delay between maximum VHG and minimum river depth was only 3–4 h.

There were few significant correlations between piezometer depth and physicochemical characteristics within the Locke Island site (i.e., $P > 0.05$) and, where they were sig-

Fig. 4. Physicochemical data collected from piezometers installed and monitored within the Locke Island site at spawning (S) and nonspawning (NS) sites during October and November 1996 and 1997. Bars above and below the points represent the 95% CI of the mean. (*a*) Hydraulic head of hyporheic water minus the hydraulic head of the river; (*b*) VHG between hyporheic and surface waters where positive values indicated potential upwelling and negative values indicated downwelling; (*c*) specific conductance of the upwelling areas (note that the specific conductance of the river was approximately 150 $\mu S \cdot cm^{-1}$); (*d*) difference in temperatures between the hyporheic and surface waters. nd, no data collected.

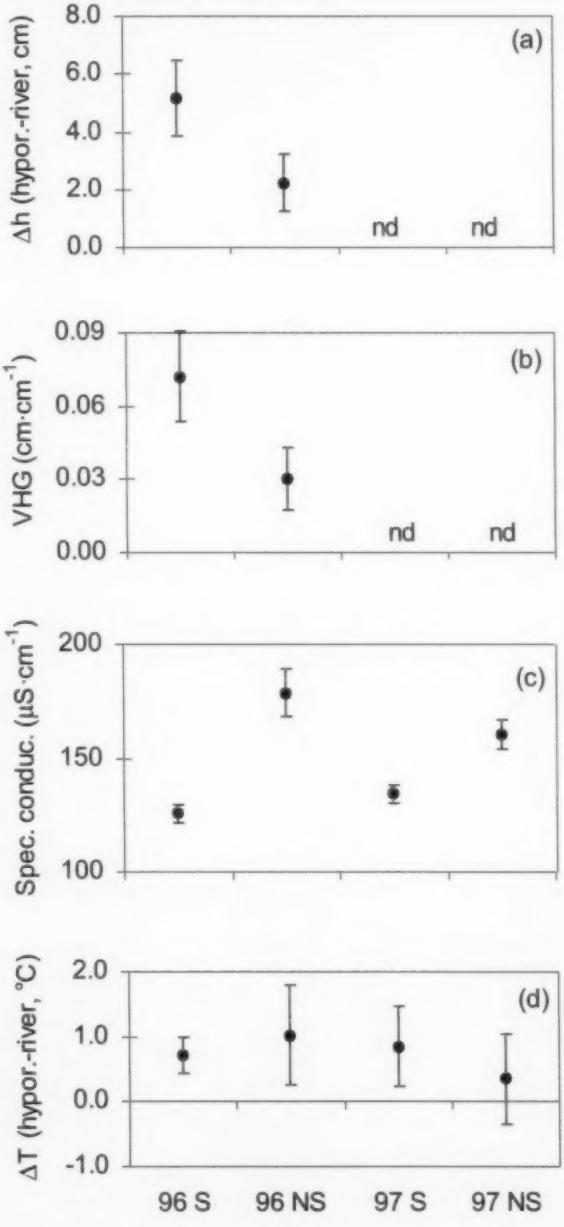


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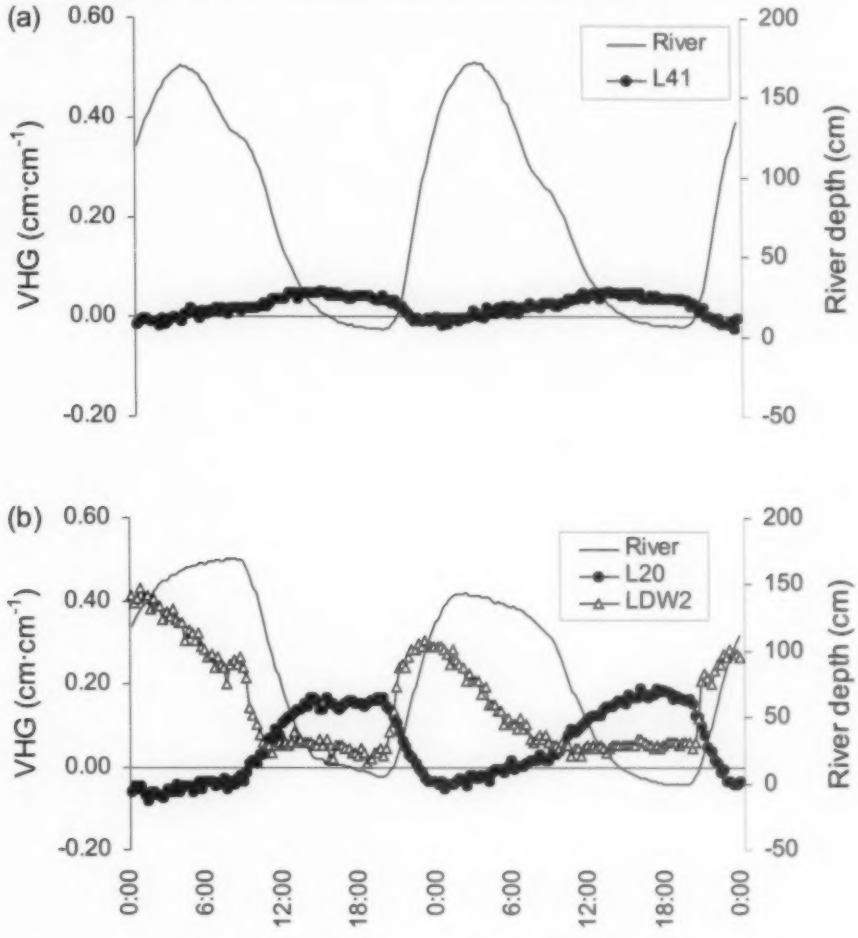
Fig. 5. Continuous measurements of VHG between hyporheic and surface waters at the (*a*) Locke Island nonspawning site (November 13–14, 1996) and (*b*) Locke Island spawning site (November 20–21, 1996). River depth represents the depth of the river within the standpipe attached to the piezometer group and is a surrogate for river discharge. The daily average discharge at Priest Rapids Dam was 2837 and 3241 $m^3 \cdot s^{-1}$ on November 13–14, 1996, and November 20–21, 1996, respectively.



nificant, the relationships were weak (i.e., $r^2 < 0.40$). The exception was in the nonspawning area in 1997 where specific conductance increased significantly with increasing distance below the riverbed ($P = 0.04$, $r^2 = 0.91$). In contrast, there was no relationship within spawning areas during that same year.

Discussion

Fall chinook salmon spawning locations were highly correlated with hyporheic discharge that was composed of mostly river water and not phreatic ground water. Hyporheic water that discharged into fall chinook salmon spawning locations was consistently greater in volume and had higher DO and lower specific conductance than discharge into nonspawning locations. This was true when comparisons were performed between a spawning and nonspawning site and also true when spawning and nonspawning clusters within a spawning site were evaluated.

That hyporheic discharge was dominated by river water suggests that sediment deposits were more permeable in the spawning areas than in the nonspawning areas. The measured hydraulic conductivities were consistent with those of riverbed sediments composed of gravel and sand (Freeze and Cherry 1979). Specific discharge of hyporheic waters was approximately three times larger at Locke Island than at Wooded Island. Repeated disruption of the substrate by fish excavating redds would likely increase the permeability of the upper 50 cm of substrate in the spawning areas above that in nonspawning areas. However, hydraulic conductivity tests showed that the permeability of the substrate was equal with increasing depth below the riverbed at Locke Island (spawning site) but not at Wooded Island (nonspawning site). This is consistent with other studies in the Locke Island area where river water has been found at depths >9 m into the substrate (R. Peterson, Pacific Northwest National Laboratory, Richland, Wash., personal communication). Thus, it is unlikely that the higher substrate permeability in

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spawning areas was solely a function of the disruption of substrate material by spawning fish.

River water was presumed to have entered highly permeable riverbed substrate at locations upstream of spawning areas. Geomorphic bed features (i.e., islands, gravel bars, riffles) of alluvial rivers are able to create hydraulic gradients sufficient to direct surface water into the bed (Stanford et al. 1996; Brunke and Gonser 1997). River water is able to penetrate deeper into hyporheic habitats if the riverbed is composed of alluvium that is highly permeable (Vaux 1962, 1968; White 1993). The more permeable the alluvium, the more that the physicochemical characteristics of the hyporheic waters will resemble surface water rather than ground water. In contrast, the relative proportion of phreatic ground water in hyporheic waters will be greater if the riverbed sediments are of low hydraulic permeability because river water will not be able to readily enter the substrate and dilute the ground water (White 1993; Brunke and Gonser 1997). This may explain the differences in specific conductance values observed between the Locke Island and Wooded Island sites.

The vertical and horizontal potentials of surface water to infiltrate into hyporheic habitats were also affected by river discharge. The diurnal fluctuations in river stage caused from power-peaking operations at Priest Rapids Dam result in layering and mixing of ground water and river water and dilution of ground water constituents and control mixing rates of ground water and surface water within riverbank hyporheic zones (Hope and Peterson 1995). Within these bank storage zones, river water is temporarily stored during periods of high river discharge and then released back to the river as discharge recedes. This alternation between hyporheic recharge and discharge was more pronounced in fall chinook salmon spawning areas than in areas of nonspawning and was presumably a result of more permeable substrate material.

It is likely that the influence of river stage changes was more pronounced along the shoreline where the piezometers were located than within the middle of the river channel. Fluctuating river stage was associated with rapid changes in VHG in nearshore piezometers, while the response was dampened in offshore areas. This is consistent with other studies that show that groundwater discharge is greatest near the shoreline interface and decreases exponentially with distance offshore (Winter 1974; Pfannkuch and Winter 1984).

While the offshore piezometers were located directly within a spawning area, there were constraints imposed by field conditions and budgets that limited how close to the spawning areas that I could place the majority of the piezometers. Consequently, most of the data were collected from piezometers that were adjacent to spawning areas or adjacent to nonspawning areas. Although the response to river stage was dampened in the offshore piezometers, the physicochemical characteristics of the offshore piezometer data compared well with values from piezometers located near the river shore. Further, inspection of aerial photographs shows that the density of fall chinook salmon spawning adjacent to piezometer clusters classified as spawning was significantly higher than adjacent to piezometers classified as nonspawning. Therefore, I am confident that the piezometers along the shore represent conditions further out in the channel with respect to the physiochemical characteristics and that differences between piezometers classified as spawning and nonspawning were real.

Hyporheic discharge was assumed to produce temperature, physical (flowing water), and (or) chemical (DO, dissolved ions) gradients in surface waters that could be used by fall chinook salmon to search for a spawning area. This assumption was based on other salmonids that use hyporheic and (or) ground water discharge to locate spawning areas. For example, brook trout (*Salvelinus fontinalis*) used discharging ion-rich water to select spawning sites in spring-fed ponds and lakes (Webster and Eiriksdottir 1976; Carline 1980; Witzel and MacCrimmon 1983; Curry and Noakes 1995) whereas chum salmon (*Oncorhynchus keta*) used temperature to locate spawning sites near ground water discharge composed of both surface and phreatic ground water (Leman 1993). In a turbid river, sockeye salmon (*Oncorhynchus nerka*) preferentially selected spawning areas near ground water discharge, but surface water temperatures of spawning sites were no different than those of nonspawning sites (Lorenz and Eiler 1989). These studies suggest that salmonids respond to hyporheic discharge, but the specific component of the discharge that provides cues is species and location specific.

There was no evidence from this study that temperature gradients between the hyporheic and surface waters provided cues for adult chinook salmon to locate spawning areas. However, the water temperature of riverbed sediments during the fall chinook salmon egg incubation period (November–March; Becker 1985) was as much as 3°C warmer than the river (D.R. Geist, unpublished data). Warm hyporheic discharge provides benefits to developing embryos by protecting the eggs from freezing and optimizing incubation and emergence periods (Curry et al. 1995). Thus, there could be a selective advantage of spawning in hyporheic discharge areas.

DO within sediments where fall chinook salmon spawned was significantly higher than DO in nonspawning areas. In both cases, the level of DO was higher than the critical levels needed for the survival of eggs and alevins (~5 $mg \cdot L^{-1}$; Bjornn and Reiser 1991). Low DO was used by brown trout (*Salmo trutta*) to avoid areas of ground water upwelling, although concentrations ranged down to 2 $mg \cdot L^{-1}$ in ground water discharge zones (Hansen 1975). In the present study, DO levels were likely a function of increased permeability of substrate within spawning areas and secondarily important in site selection by prespawning fish.

The ability of fish to detect gradients between hyporheic and surface waters will be a function of gradient intensity as determined by the rate of upwelling (Curry and Noakes 1995). The amount of upwelling in spawning areas at Locke Island was an order of magnitude higher than the amount of upwelling at the Wooded Island site and in nonspawning areas at the Locke Island site, although at both sites, the quantity of hyporheic discharge was <1% of the discharge of the surface water. Thus, an extremely sensitive sensory mechanism would be required for fall chinook salmon to use hyporheic discharge to locate spawning areas.

Chemical differences between organic and inorganic constituents of ground water and surface water may provide cues that sexually mature fall chinook salmon use for spawning site discrimination. Sex hormones (e.g., estradiol-17β,

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testosterone) cause hypersensitivity of the olfactory system during sexual maturation in anadromous salmonids (Hasler and Wisby 1951; Hasler and Scholz 1983). Based on electrophysiological experiments, the olfactory system of salmon can differentiate concentrations of some organic chemicals on the order of 1×10^{-7} M (Hara 1970). Thus, it is conceivable that even at relatively low quantities of hyporheic discharge, chemical cues resulting from hyporheic discharge are detectable by Hanford Reach fall chinook salmon. However, additional information would be needed before a conclusion could be drawn on the relative importance of chemical cues in redd site selection by fall chinook salmon in the Hanford Reach.

Current salmon restoration efforts are investigating salmon recovery options on the mainstems of large rivers, including dam removal, reservoir drawdown, and alterations in dam operations. Before these options are implemented, additional information on the life history requirements of salmon that utilize these habitats is needed. The results from this study suggest that hyporheic discharge helped explain aspects of fall chinook salmon redd patterns previously not explained by physical (hydraulic) models. The incorporation of measures of ground water – surface water interactions into current habitat-use models would provide managers a better definition of fall chinook salmon spawning habitat that would likely result in better predictions of recovery potential and more efficient use of limited recovery dollars.

Acknowledgments

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Use of fish production per unit biomass ratios for measuring the productive capacity of fish habitats

R.G. Randall and C.K. Minns

Abstract: Species-specific production rate per unit biomass (*P/B*, per year) ratios were calculated for 79 freshwater fish species of eastern Canada. *P/B* (per year) ratios were calculated using two methods, which were based on allometry with fish weight-at-maturity and life expectancy, respectively. *P/B* (per year) values obtained by the two methods were significantly correlated, as expected from life history theory, since the two predictors (longevity, size-at-maturity) were themselves correlated. Species-specific *P/B* (per year) ratios were also significantly correlated with field observations of *P/B* from published sources. The estimation of *P/B* based on allometry with fish size is recommended because of its utility; the predictive equation is *P/B* (per year) = $2.64W_{mat}^{-0.35}$, where W_{mat} is weight-at-maturity. Both the coefficient 2.64 and the exponent –0.35 of this equation are provisional and require further validation from field studies of fish production. More accurate estimates of *P/B* are possible if population-specific information on size-at-maturity or mean size is known. The product of average fish biomass and estimated *P/B* coefficients (habitat productivity index (HPI) = $B \times P/B$) is a proposed measure of habitat productive capacity.

Résumé : Nous avons calculé les rapports spécifiques à l'espèce *P/B* (an^{-1}) (taux de production par unité de biomasse) pour 79 espèces de poissons dulcicoles de l'est du Canada. Les rapports *P/B* (an^{-1}) ont été calculés par deux méthodes, fondées sur l'allométrie avec respectivement le poids à la maturité du poisson et l'espérance de vie. Les valeurs de *P/B* (an^{-1}) obtenues par les deux méthodes étaient significativement corrélées, comme on pouvait le prévoir théoriquement, car deux prédicteurs (longévité, taille à la maturité) étaient eux-mêmes corrélés. Les rapports spécifiques *P/B* (an^{-1}) étaient aussi significativement corrélés aux observations de terrain fournies par diverses sources publiées. Nous recommandons l'estimation de *P/B* basée sur l'allométrie avec la taille des poissons, du fait de son utilité; l'équation prédictive est : *P/B* (an^{-1}) = $2{,}64W_{mat}^{-0.35}$, W_{mat} correspondant au poids à la maturité. Le coefficient 2,64 et l'exposant –0,35 sont provisoires et doivent être validés par des travaux de terrain sur la production des poissons. Il est possible d'obtenir des estimations plus précises de *P/B* si on dispose d'information spécifique à la population sur la taille à la maturité ou sur la taille moyenne. Nous proposons, pour mesurer la capacité productive d'un habitat, de faire le produit de la biomasse moyenne de poisson et des coefficients estimés de P/B (indice de productivité de l'habitat = $B \times P/B$).

[Traduit par la Rédaction]

Introduction

Fish production is the best indicator of the quantitative performance of a fish population in any type of habitat, and it is a measure of productive capacity (Jones et al. 1996; Minns et al. 1996). However, estimation of production in the field is time consuming and costly, and often, surrogates of production are used to evaluate habitat suitability or capacity. For any defined time period, fish production is the product of average biomass and specific growth rate, and therefore, fish biomass and fish production are correlated (Downing et al. 1990; Downing and Plante 1993; Randall et al. 1995). Animal biomass is sometimes used as a surrogate of production (Banse and Mosher 1980). For freshwater fish, the slope of the regression of production versus biomass is not significantly different from unity, indicating that specific production does not vary systematically with biomass, and therefore, fish production can be approximated by using an average production to biomass ratio (Downing and Plante 1993). Specific or population production (i.e., production per unit biomass, *P/B*) varies with fish size, however, and a more accurate estimate of production is possible if both biomass and fish size are used as predictors (Boudreau and Dickie 1989; Downing and Plante 1993; Randall et al. 1995). Fish growth and mortality are related to fish size (Peters 1983), and consideration of fish size accounts for some of the variability in specific production.

Specific production (*P/B*) considered in this paper is based on a 1-year time frame (annual production divided by mean annual biomass), and it applies to populations in steady state (not increasing or decreasing in abundance with time). The *P/B* (per year) ratio indicates how quickly biomass is potentially changing. For fish populations in lakes, most *P/B* ratios varied between 0.2 and 5.0, and were inversely related to maximum size of the fish in the populations and positively related to lake productivity (Downing et al. 1990; Downing and Plante 1993). Most *P/B* ratios were within this range for fishes inhabiting rivers as well (Mann and Penczak 1986). In a population dynamic context, maxi-

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R.G. Randall[1] and C.K. Minns. Department of Fisheries and Oceans, P.O. Box 5050, 867 Lakeshore Road, Burlington, ON L7R 4A6, Canada.

[1]Author to whom all correspondence should be addressed.
e-mail: randallr@dfo-mpo.gc.ca

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Table 1. Life history characteristics of 79 species of fish that inhabit the freshwaters of eastern Canada.

Species	Code	L_{mat} (SL, mm)	L_{max} (SL, mm)	T_{mat} (years)	T_{max} (years)	W_{mat} (g)	W_{max} (g)	Method	P/B_Z	P/B_W	P/B
Longnose gar	F0041	500	1830	6	32	1945.3	78 548.0	LMAT	0.14	0.09	0.19
Bowfin	F0051	610	870	4	25	3055.4	8 977.4	LW	0.18	0.08	0.16
Alewife	F0061	138	205	3	6	36.6	108.8	LW	0.73	0.37	0.75
Gizzard shad	F0063	279	521	2	6	472.5	3 100.4	LW	0.73	0.15	0.31
Pink salmon	F0071	411	508	2	2	1105.3	2 057.38	LMAT	2.14	0.11	0.23
Coho salmon	F0073	635	900	3	3	3875.7	10 452.2	LMAT	1.43	0.07	0.15
Chinook salmon	F0075	750	968	3	9	5463.0	11 550.8	LW	0.49	0.06	0.13
Rainbow trout	F0076	349	915	4	8	681.5	12 331.6	LW	0.55	0.13	0.27
Atlantic salmon	F0077	800	1550	3	5	7545.0	48 997.3	LMAT	0.87	0.06	0.12
Brown trout	F0078	258	874	3	18	361.6	11 505.4	LW	0.25	0.17	0.34
Brook trout	F0080	156	350	3	9	52.9	627.1	LWL	0.49	0.33	0.66
Lake trout	F0081	510	1310	5	20	2059.6	30 376.3	LMAT	0.22	0.09	0.18
Lake whitefish	F0091	236	526	4	17	229.2	2 771.4	LWL	0.26	0.20	0.39
Lake herring	F0093	246	395	3	13	251.5	1 006.4	LMAT	0.34	0.19	0.38
Round whitefish	F0102	183	468	3	14	107.2	1 629.6	LMAT	0.32	0.26	0.51
Rainbow smelt	F0121	125	297	3	6	15.7	192.9	LW	0.73	0.50	1.01
Northern pike	F0131	534	1000	3	12	1349.8	8 889.8	LW	0.37	0.11	0.21
Muskellunge	F0132	650	1426	4	22	4145.6	38 659.6	LMAT	0.20	0.07	0.14
Grass pickerel	F0133					9.7	75.3	WMAX		0.60	1.19
Central mudminnow	F0141	110	132	1	4	24.7	44.7	LMAT	1.08	0.43	0.86
Quillback	F0161	472	660	6	12	2501.8	7 615.4	LW	0.37	0.09	0.17
Longnose sucker	F0162	240	583	6	24	234.3	3 042.8	LMAT	0.19	0.20	0.39
White sucker	F0163	253	487	4	15	280.3	1 983.4	LW	0.30	0.18	0.37
Chubsucker	F0164					15.9	128.3	WMAX		0.50	1.00
Silver redhorse	F0168	391	635	5	14	957.2	3 879.1	LMAT	0.32	0.12	0.24
Shorthead redhorse	F0171	195	620	3	14	138.7	4 818.8	LW	0.32	0.23	0.47
River redhorse	F0173					147.0	1 400.5	WMAX		0.23	0.46
Goldfish	F0181	175	457	4	30	274.4	2 384.7	LW	0.15	0.18	0.37
Redbelly dace	F0182	46	61	1	8	2.0	5.0	LMAT	0.55	1.03	2.07
Finescale dace	F0183	59	80	2	8	4.1	10.8	LMAT	0.55	0.80	1.61
Lake chub	F0185	108	189	3	5	23.4	123.9	LMAT	0.87	0.44	0.88
Carp	F0186	353	800	4	20	1199.7	13 424.8	LW	0.22	0.11	0.22
Brassy minnow	F0189	57	158	1	4	3.7	74.4	LMAT	1.08	0.83	1.67
Hornyhead chub	F0192	93	188	3	4	15.2	122.0	LMAT	1.08	0.51	1.02
Golden shiner	F0194	64	211	2	7	4.2	164.6	LW	0.62	0.80	1.59
Emerald shiner	F0196	42	124	1	4	0.9	21.3	LW	1.08	1.38	2.76
Common shiner	F0198	74	169	1	5	7.9	90.1	LMAT	0.87	0.64	1.28
Blackchin shiner	F0199	37	71	1	4	0.9	4.6	LW	1.08	1.37	2.73
Blacknose shiner	F0200	24	81	1	3	0.3	11.2	LMAT	1.43	2.00	4.00
Spottail shiner	F0201	55	137	1	4	2.6	38.8	LW	1.08	0.94	1.89
Mimic shiner	F0206	35	63	1	2	0.9	5.5	LMAT	2.14	1.36	2.73
Bluntnose minnow	F0208	50	78	1	3	1.7	7.3	LW	1.43	1.10	2.21
Fathead minnow	F0209	54	73	1	4	4.9	9.7	LWL	1.08	0.76	1.51
Blacknose dace	F0210	49	58	2	3	2.4	4.3	LMAT	1.43	0.97	1.94
Longnose dace	F0211	74	118	2	5	7.9	32.5	LMAT	0.87	0.64	1.28
Creek chub	F0212	87	240	2	5	13.6	185.0	LWL	0.87	0.53	1.06
Fallfish	F0213		420			140.5	1 198.2	LMAX		0.23	0.47
Pearl dace	F0214	94	132	2	4	15.9	37.6	LWL	1.08	0.50	1.00
Brown bullhead	F0233	161	297	3	8	79.5	488.9	LW	0.55	0.29	0.57
Channel catfish	F0234	337	1004	7	24	623.5	14 261.9	LMAT	0.19	0.14	0.28
Tadpole madtom	F0236	62	110	1	3	4.7	26.6	LMAT	1.43	0.77	1.53
American eel	F0251	457	1520	9	27	462.3	4 390.5	LW	0.17	0.15	0.31
Banded killifish	F0261	53	102	2	3	3.0	21.5	LMAT	1.43	0.95	1.80
Burbot	F0271	370	775	3	13	816.3	6 833.5	LMAT	0.34	0.13	0.25
Brook stickleback	F0281	50	87	1	3	2.5	13.7	LMAT	1.43	0.95	1.90
Threespine stickleback	F0282					0.5	2.9	WMAX		1.72	3.45
Ninespine stickleback	F0283	35	68	2	3	0.9	6.8	LMAT	1.43	1.36	2.73

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Table 1 (*concluded*).

Species	Code	L_{mat} (SL, mm)	L_{max} (SL, mm)	T_{mat} (years)	T_{max} (years)	W_{mat} (g)	W_{max} (g)	Method	P/B_Z	P/B_W	P/B
Fourspine stickleback	F0284	41	52	2	2	1.4	3.2	LMAT	2.14	1.16	2.33
Trout-perch	F0291	78	123	2	4	6.5	51.7	LW	1.08	0.69	1.37
White perch	F0301	254	482	2	12	412.1	3 067.7	LW	0.37	0.16	0.32
White bass	F0302	115	322	3	8	47.0	684.6	LW	0.55	0.34	0.69
Rock bass	F0311	54	222	2	10	4.9	338.7	LW	0.44	0.75	1.51
Green sunfish	F0312	76	169	2	9	8.5	90.1	LMAT	0.49	0.62	1.25
Pumpkinseed	F0313	59	219	2	9	6.5	391.2	LW	0.49	0.68	1.37
Bluegill	F0314	93	250	3	9	25.3	502.2	LW	0.49	0.43	0.85
Smallmouth bass	F0316	264	423	4	12	380.8	1 509.0	LW	0.37	0.16	0.33
Largemouth bass	F0317	272	453	3	15	429.0	2 014.6	LW	0.30	0.16	0.32
Black crappie	F0319	192	300	3	9	150.5	563.7	LW	0.49	0.23	0.46
Yellow perch	F0331	175	334	4	10	96.1	650.0	LW	0.44	0.27	0.53
Sauger	F0332	283	593	4	13	376.8	3 193.5	LMAT	0.34	0.17	0.33
Walleye	F0334	328	641	4	20	527.7	3 998.7	LW	0.22	0.15	0.29
Iowa darter	F0338	43	58	1	3	1.6	4.3	LMAT	1.43	1.11	2.22
Johnny darter	F0341	37	60	1	4	1.1	4.8	LMAT	1.08	1.29	2.58
Logperch	F0342	106	150	2	3	14.9	39.6	LW	1.43	0.51	1.03
Tessellated darter	F0346	43	77	1	4	1.6	9.7	LMAT	1.08	1.11	2.22
Brook silverside	F0361	74	81	1	2	7.9	11.2	LMAT	2.83	0.64	1.28
Freshwater drum	F0371	292	940	5	17	393.6	19 172.6	LW	0.26	0.16	0.33
Mottled sculpin	F0381	60	82	2	4	4.3	11.5	LMAT	1.08	0.79	1.58
Slimy sculpin	F0382	63	120	3	7	5.0	34.1	LMAT	0.62	0.75	1.51

Note: Code, unique identification for each species; Method, method for estimating W_{mat} and W_{max} (LW, weight estimated using length–weight coefficients from the productive capacity database (Appendix 1); LWL, length–weight coefficients from Appendix 2; LMAT, weight estimated from eq. 3 or 4 (Table 2); WMAX, W_{mat} estimated from eq. 2; LMAX, weight estimated from eqs. 1, 3, and 4); P/B_Z and P/B_W, ratio of production to biomass as estimated from longevity and weight-at-maturity, respectively; P/B, production to biomass ratio based on weight-at-maturity, with an adjusted coefficient (see text).

mum P/B is equal to the maximum rate of population increase (R, i.e., $P/B_{max} = R_{max}$ (Peters 1983)). Rate of population growth (and P/B) can be used to estimate the amount of time required for a population to establish itself after a catastrophic decline, to colonize a new habitat, or to withstand exploitation (Peters 1983; Charnov 1993). Both P/B and R_{max} are expressions of growth potential (biomass and numbers) and are linked to the productive capacity of the habitat.

As will be seen, the P/B ratio varies inversely with fish size-at-maturity and longevity and is therefore specific for species and populations. P/B has been calculated for species based on their life span (Waters 1977), body size (Dickie 1972; Banse and Mosher 1980), and life history growth and mortality patterns (Allen 1971; Waters 1977; van Straalen 1985). The objective of this study was to calculate species-specific P/B (per year) ratios for several species of fishes ($n = 79$) that inhabit the freshwaters of eastern Canada. P/B (per year) ratios are calculated and compared using two methods that are based on allometry with fish size and longevity. The generalized species-specific P/B (per year) ratios are compared with actual field estimates (literature) for a subset of the species. Finally, a potential application for the use of species-specific P/B ratios to evaluate habitat suitability and capacity is proposed and examined.

Methods

Fish database sources

Three databases were used to obtain species-specific information on life history traits. Fish lengths were given as standard length (SL), fork length (FL), or total length (TL) in millimetres, depending on the database (see below). Where necessary, TL and FL were converted to SL by dividing by 1.2 and 1.1, respectively (Portt et al. 1991). Weights are in grams.

Portt et al. (1991) and Minns et al. (1993) summarized the life history characteristics of 75 species of freshwater fishes that inhabit inland lakes in Ontario. Hereafter, the Portt et al. (1991) and Minns et al. (1993) database is referred to as the LakeDB. Species-specific length-at-maturity (L_{mat}) (SL, millimetres), maximum length (L_{max}) (SL, millimetres), age-at-maturity (T_{mat}, years), and maximum age (T_{max}, years) from the LakeDB were used for this study. Recognizing that these traits are population dependent, the authors used medial values for each species when possible (for further details, see Minns et al. 1993).

Parameters a and b for the length–weight regression $W = aL^b$ for a subset of the species were derived from a productive capacity fish database, which was based on electrofishing samples from the lower Great Lakes (Moore et al. 1998) (hereafter referred to as the CapacityDB). Species for which at least 10 individuals were measured for length (usually FL; Valere 1996) and for which the coefficient of determination was >0.50 were used (Appendix 1). The total number of species meeting these criteria was 36, of which four were found in the CapacityDB but not in the LakeDB. The total number of freshwater species considered in this study was therefore 79. Length–weight data for an additional five species (used for validation, see below) were obtained from the literature (Appendix 2). The length–weight parameters were used to convert the LakeDB length data (maturity and maximum) to weights. For the remaining species for which the length–weight coefficients were not known ($n = 38$), weight-at-maturity (W_{mat}) or maximum weights (W_{max}) were estimated by regression (Table 1). The CapacityDB was also used to estimate the L_{max} of fish for each

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taxon, based on the arithmetic average of the 10 largest individuals in the sample (Pauly 1984). Maximum fish lengths from CapacityDB were compared with the maximum sizes reported by Portt et al. (1991) and Minns et al. (1993) to test for consistency in the species size data between data sets.

For a subset of species, asymptotic lengths (L_{inf}) and asymptotic weights (W_{inf}) were obtained from a published database (FishBase 98; Froese and Pauly 1998). These parameters were available for 142 populations of 28 species (Froese and Pauly 1998). Maximum size of fish species in the LakeDB was compared with these asymptotic sizes.

Estimation of *P/B* ratios

Data from the three databases were used to estimate species-specific *P/B* ratios using the life history information on fish W_{mat} (grams) and longevity (years).

Allometric theory based on animal energetics predicts that *P/B* declines with weight as $W^{-0.25}$ (Lavigne 1982). However, Banse and Mosher (1980) showed that the weight exponent was significantly different from –0.25 within taxonomic groups with similar production efficiencies; they found an exponent of –0.37. Other researchers have also found coefficients that were different than expected from allometric theory (Dickie et al. 1987; Boudreau and Dickie 1989; Randall et al. 1995). Dickie et al. (1987), Boudreau and Dickie (1989), and Boudreau et al. (1991) showed that the different slopes were related to the influence of both primary (physiological) and secondary (ecological) scaling factors. A regression (log *P/B* versus log *W*) across taxonomic groups (e.g., invertebrates and fish) gave a lower slope (about –0.2) than a regression within a taxonomic group (e.g., fish with a slope of about –0.3) (see fig. 1*a* and table 1 in Boudreau and Dickie 1989). The secondary scaling factor was related to predator–prey interactions and energy transfer between trophic positions (Thiebaux and Dickie 1992, 1993). For this paper, *P/B* was estimated using an allometric equation developed by Randall et al. (1995) for fish inhabiting lakes and rivers: $\log P/B = 0.12 - 0.35 \log W_{mat} + 0.18H$, where W_{mat} is the weight-at-maturity (grams) and H is a habitat indicator variable (0 for lakes, 1 for rivers). (Note that in the original paper, Randall et al. (1995) used average fish weight of fish in the community rather than W_{mat}.) The partial slope of –0.35 with W_{mat} in this regression is consistent with the exponent of the within-fish subgroup discussed above. Retransformed assuming a lake habitat gives the allometric formula $P/B_W = 1.32W_{mat}^{-0.35}$. The estimate of P/B_W was applied to all 79 species of fishes.

Hoenig (1983) demonstrated that plots of total mortality versus T_{max} were negatively correlated for three taxonomic groups, mollusks, fishes, and cetaceans, and could be described by the predictive equation $\ln Z = 1.44 - 0.982 \ln T_{max}$, where Z is the instantaneous mortality rate (annual) and T_{max} is the maximum age in the population. Mortality rate is inversely related to longevity. Parameters for the regression were similar for all three groups of animals, and Hoenig recommended that the combined data set be used for prediction. For balanced populations, annual *P/B* is approximately equal to instanteous mortality, if mortality is constant with age (Dickie 1972; van Straalen 1985; Mertz and Myers 1998), i.e., $P/B = Z$, and therefore, the Hoenig equation can also be used as a first-order estimate of population production (reexpressed in a nonlog form: $P/B_Z = 4.22T_{mat}^{-0.982}$). The estimate of P/B_Z was applied to 75 of 79 species for which T_{max} was known. The two estimates of *P/B* (per year) were compared by correlation analysis (Pearson product-moment) and were validated by interspecies correlation with field estimates of *P/B* from Downing and Plante (1993).

Results

Life history characteristics

The L_{max} varied among species from 52 to 1830 mm (Table 1). Many of the species were small and short-lived; about 70% of the 79 species matured at age 3 or less.

The L_{mat} was related to L_{max} as described by the equation $\log L_{mat} = -0.005 + 0.880 \log L_{max}$ ($n = 74$, $F = 642.2$, $R^2 = 0.90$, $P < 0.001$) (Fig. 1; Table 2). The L_{max} and L_{inf} predicted from the von Bertalanffy growth equation should be reasonably close (say ±30%; Froese and Pauly 1998). In conformity with this, L_{max} (LakeDB) and L_{inf} (FishBase98) were significantly correlated (Fig. 2), confirming that L_{max} provided an approximation of L_{inf} and that the sizes in the LakeDB were approximately medial values for each species (Minns et al. 1993). The corresponding equation for the relationship between W_{mat} and W_{max} for the subset of species was $\log W_{mat} = -0.762 + 0.931 \log W_{max}$ ($n = 38$, $F = 191.6$, $R^2 = 0.84$, $P < 0.0001$) (Table 2).

Species-specific L_{max} values from the LakeDB were also significantly correlated with maxima for 35 species calculated from field data collected from the Great Lakes in the CapacityDB (Fig. 3). This result was consistent with the hypothesis that maximum size can be estimated from field samples by using the 10 largest individuals in large samples (Pauly 1984). Together, Figs. 2 and 3 confirmed that all three databases were consistent and provided roughly similar trends in maximum size.

P/B ratios

For the 79 species of fishes, *P/B* (per year) ratios as calculated by the two methods were significantly correlated (Fig. 4). The average *P/B* values were least for P/B_W and highest for P/B_Z (Table 3). Most of the values for P/B_Z were within the bounds of 0.2 and 5, consistent with field observations of fish biomass and production in lakes around the world (Downing and Plante 1993). However, this was not the case for the P/B_W estimates, which were lower (37% were less than 0.2). The slope of $\log P/B$ on $\log W_{mat}$ was –0.19 for P/B_Z, which was different from the slope of –0.35 used for P/B_W (Table 3).

P/B ratios estimated by the two methods were correlated with published information on *P/B* ratios for fish species in lakes (Downing and Plante 1993). A subset of data from Downing and Plante (1993), which included nine species that were also found in our database, were used for this comparison. The subset included 24 populations from nine lakes in Canada; average biomass (kilograms per hectare), annual production (kilograms per hectare per year), and *P/B* (per year) ratios were given for each population. Both the P/B_Z and P/B_W estimates were correlated with the observed ratios in the published field studies (Table 4; where more than one estimate of *P/B* was available for three of the nine species, an average was used). Although the sample size was small ($n = 9$ species), these results suggested that the species-specific estimators of P/B_Z and P/B_W were useful predictors of actual *P/B* ratios. Both methods, however, underestimated on average the actual specific production coefficients as measured in the field.

Theoretically, *P/B* estimates based on population size-at-maturity data would be more accurate than the above estimates that are based on species "average" size-at-maturity data. For the published data on fish production in lakes, Downing and Plante (1993) also reported the maximum individual size of fish in the populations. Population size-at-

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Fig. 1. Scatterplot matrices of life history traits of the species of fishes listed in Table 1. Each point represents one of 79 species. The traits are L_{mat} (SL), L_{max} (SL), T_{mat}, T_{max}, and Z. Species length and age data were from Portt et al. (1991) and Minns et al. (1993). Mortality was estimated from T_{max} (see text). Data are plotted on a $\log_{10}$ scale.

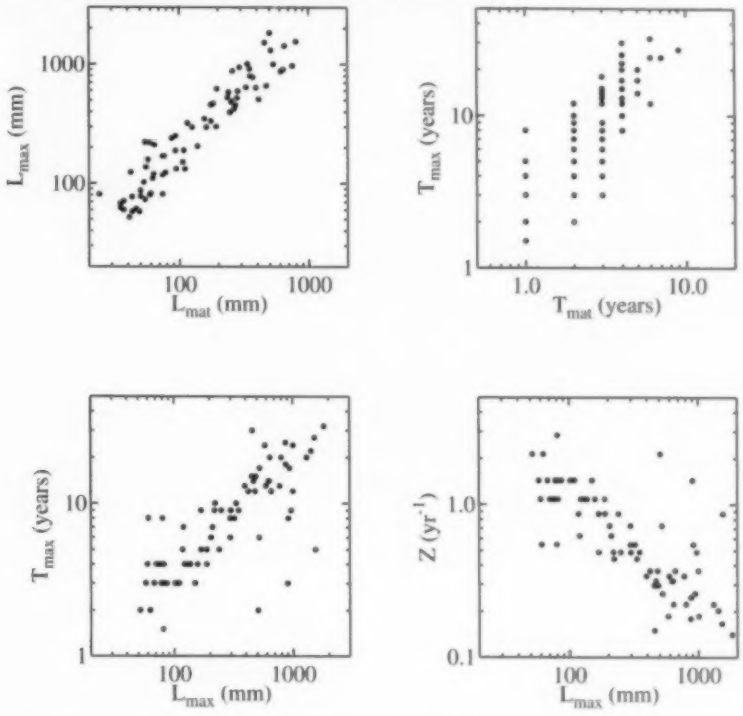

Table 2. Regressions used to estimate W_{mat} and W_{max} in Table 1.

Equation	Dependent	Independent	n	F statistic	Intercept	Slope	R^2
1	log L_{mat}	log L_{max}	74	642.2	–0.005	0.880	0.90
2	log W_{mat}	log W_{max}	38	191.6	–0.762	0.931	0.84
3	log W_{mat}	log L_{mat}	38	1098.1	–4.491	2.884	0.97
4	log W_{max}	log L_{max}	38	625.6	–4.372	2.842	0.94

maturity for 16 of the 24 populations (maximum size was constant in eight populations) was estimated using the regression of W_{mat} on W_{max} (Table 2, eq. 2). Coefficients of determination for the regression of P/B on species and population W_{mat} for the 16 populations were $R^2 = 0.65$ and $R^2 = 0.62$, respectively, and thus, there was little difference in the predictors in this case. This was likely due to the significant correlation between the species average and the population-specific maturity weights for the species in this data set ($n = 16$, $F = 27.7$, $R^2 = 0.67$, $P < 0.0001$).

For the 24 populations, production rate was estimated as the product of the reported biomass (kilograms per hectare from Downing and Plante 1993) and the species-specific P/B_W ratios that were determined in this study. The correlation between the expected and observed production rates was highly significant ($n = 24$, $F = 61.8$, $R^2 = 0.74$, $P < 0.0001$) (Fig. 5). One species (*Perca flavescens*) was a significant outlier (studentized residual = 4.26) in this regression. The coefficient of determination for the regression of biomass alone on production for the 24 populations was 0.65, and therefore, the precision of the prediction increased by 9% (from 0.65 to 0.74, $n = 24$) if a species-specific rather than an average P/B ratio was used. First-order approximations of production of a variety of fish species can be estimated from information on field biomass and species P/B ratios.

Discussion

Species-specific P/B (per year) ratios are potentially useful to fisheries biologists for determining first-order estimates of production from field biomass data. The following topics that relate to the application and limitations of the P/B coefficients developed in this study are discussed below: characteristics of the species database, validation of the species-specific P/B ratios, and finally the potential use of P/B coefficients for determining habitat suitability and productive capacity from field estimates of fish biomass.

Database characteristics and limitations

The P/B (per year) ratios that have been generated are applicable to freshwater fish populations inhabiting the differ-

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Fig. 2. Correlation between L_{max} (SL) as reported in the LakeDB (Portt et al. 1991; Minns et al. 1993) and L_{inf} (SL) as reported for a number of species and populations in FishBase 98 (Froese and Pauly 1998). The vertical row of circles for some of the species values of L_{max} shows the range of L_{inf} values reported for individual populations (Froese and Pauly 1998). The broken line is the least-squares regression and the solid line shows the 1:1 relationship.

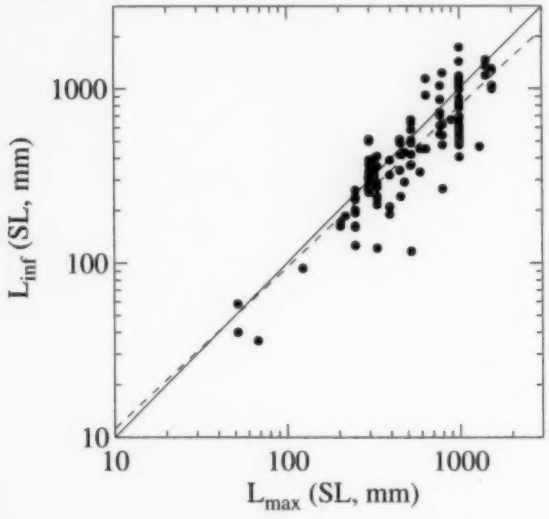
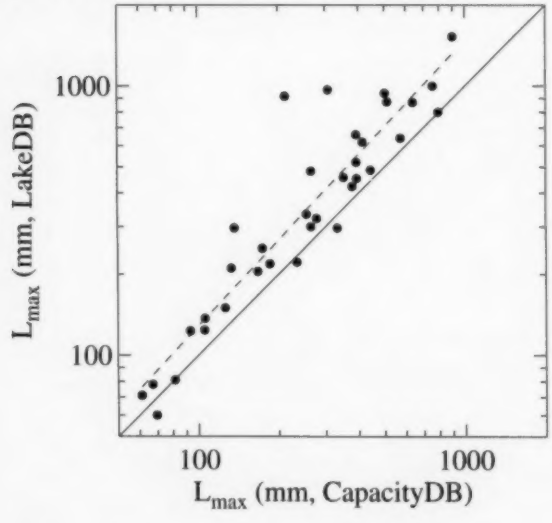


Fig. 3. Comparison of L_{max} (SL) as estimated as the average of the 10 largest individuals in the CapacityDB and as reported for 35 species in the LakeDB. The broken line is the least-squares regression and the solid line shows the 1:1 relationship.



ent areas of eastern Canada. Data on life history parameters summarized by Portt et al. (1991) and Minns et al. (1993) were originally collated because of an interest in the taxocene assemblage structure of the fish species commonly found in inland lakes in Ontario. Additional growth data (length–weight) were available for several of the same species from samples collected from the lower Great Lakes. Many of the 79 species also occur elsewhere in eastern Canada, and some occur in both lake and river habitats. Zoogeographically, all of these species are listed as part of the Atlantic drainage basin in eastern Canada (Scott and Crossman 1973), and they include many of the common species that inhabit this Atlantic region. The 79 species comprised 48% of the 165 species reported for Ontario (Mandrak and Crossman 1992) and a large proportion of the freshwater species found in Quebec, Newfoundland, and the Maritime Provinces. The species life history traits in Table 1 are not unique to Ontario populations.

Fig. 4. Comparison of P/B ratios for 79 species of fish as estimated from mortality (P/B_Z) and allometry with weight-at-maturity (P/B_W, using the adjusted coefficient of 2.64; see text). Axes are on a $\log_{10}$ scale.

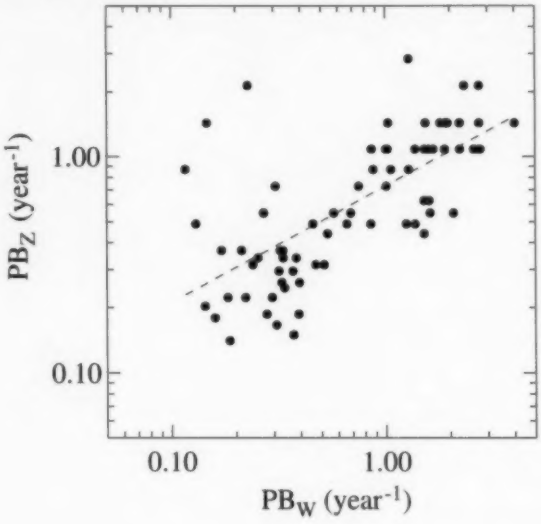


Table 3. Comparison of P/B ratios calculated from information on longevity (P/B_Z) and fish weight-at-maturity (P/B_W).

Method	Average	Range	Percent	Slope (95% CL)
P/B_Z	0.8	0.1–2.8	92	–0.19 (–0.14, –0.24)
P/B_W[a]	0.5	0.1–2.0	63	–0.35 (–0.23, –0.47)
P/B_W[b]	1.1	0.1–4.0	90	

Note: Percent is the percentage of values between 0.2 and 5.0 (see text). The slope of the regression of log P/B on log weight-at-maturity is indicated.

[a]Slope and 95% CL from Randall et al. (1995).

[b]P/B calculated using the adjusted proportionality coefficient (see text). The slope remains as –0.35.

Several of the life history traits covaried among species, as expected from life history theory. Comparison of the correlations among traits from this study with the literature-based expectations is informative. The ratio L_{mat}/L_{inf} is considered to be one of three life history invariants (Charnov 1993). The ratio of L_{mat}/L_{max} ($\equiv L_{mat}/L_{inf}$) of 0.53 (95% confidence limits (CL) = 0.5–0.6), determined by the arithmetic average using the data in Table 1, was within the range of

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Table 4. Comparison of P/B of nine species of fishes as estimated from allometry with weight-at-maturity (P/B_W) and longevity (P/B_Z) and as observed in field populations (from Downing and Plante 1993).

Species	T_{mat}	T_{max}	P/B_W	P/B_Z	P/B_{field}
Lake whitefish	4	17	0.39	0.26	0.21 (0.11–0.31, 8)
Brook trout	3	9	0.66	0.49	0.82 (0.55–2.00, 7)
White sucker	4	15	0.37	0.30	0.23 (0.17–0.31, 3)
Creek chub	2	5	1.06	0.87	2.50
Fathead minnow	1	4	1.51	1.08	2.87
Golden shiner	2	7	1.59	0.62	2.00
Yellow perch	4	10	0.53	0.44	2.52
Pearl dace	2	4	1.00	1.08	2.08
Walleye	4	20	0.29	0.22	0.36

Note: Values of P/B_{field} for the first three species are averages with ranges and the number of populations, respectively, in parentheses. For the remaining species, only one population was studied.

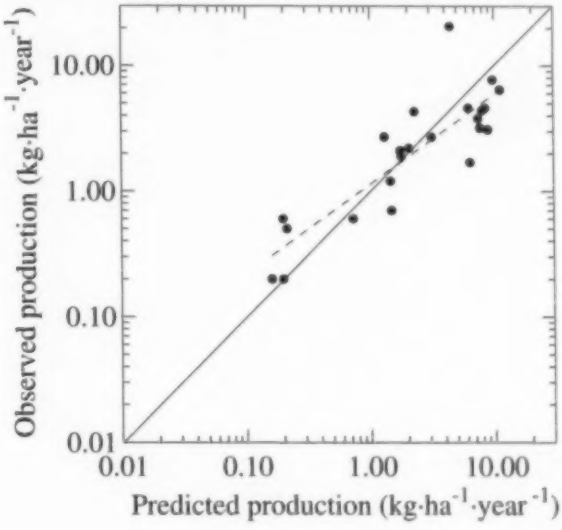
0.4–0.8 for L_{mat}/L_{inf} observed for fishes in general (Beverton and Holt 1959; Charnov 1993), although the overall ratio was lower than that predicted by ecological theory (L_{mat}/L_{inf} constant of 0.66; Jensen 1996). The average value of $Z \times T_{mat}$ indicated a product of 1.6 (95% CL = 1.4–1.8), which was similar to the theoretical value of 1.65 (Jensen 1996) but was less than the range of 1.75–2.2 reported for "typical fish" by Charnov (1993). Generally, the life history traits were correlated as expected from life history theory. The reason for the lower values of L_{mat}/L_{max} and $Z \times T_{mat}$ in our data set compared with some reported values is unknown, although it may be related to the taxonomic diversity of our species database, as noted below.

The above comparison of life history traits among species has limitations. Normally, the life history traits are considered to be constants within taxonomic groups (Charnov 1993). Some of the variability between L_{mat} and L_{max}, for example, was probably attributable to the diversity of taxonomic groups and species that were considered in Table 1. The relationship may be less variable if the same taxonomic group was considered (e.g., salmonids or cyprinids). Despite these limitations, the covariance among life history traits was useful for determining W_{mat} and first-order P/B coefficients for the diverse group of species considered in this study.

Estimates of species-specific P/B ratios can be applied to population biomass, but they will be approximations of specific production. Life history traits vary among populations of the same species. Variability in L_{inf} among populations in different habitats, for example, is clearly illustrated in Fig. 2. Variability in traits among populations is particularly evident for long-lived species of fishes. Shuter et al. (1998) found a twofold variation in L_{inf} and a fourfold variation in Z for lake trout (*Salvelinus namaycush*) populations in Ontario, and they cited similar variation in size and mortality traits for walleye (*Stizostedion vitreum*) populations. Applying a species constant P/B to specific populations is therefore an approximation. The potential error will be highest for long-lived species and least for short-lived species.

Nevertheless, we advocate the use of generic species P/B coefficients, in the absence of population-specific information on life history traits. If population-specific traits (such as W_{mat}) are known for a particular population and area, they should be used to estimate P/B (using the allometric equation) rather than the generic species P/B coefficients.

Fig. 5. Comparison of predicted versus observed production for 24 populations of freshwater fishes. Predicted production was estimated as the product of population biomass (from Downing and Plante 1993) and species-specific P/B_W (where $P/B_W = 2.64W^{-0.35}$; see text). The broken line is the regression line and the solid shows the 1:1 relationship. The highest observed production value was an outlier (see text).



Validation and calibration

P/B ratios can be validated by intercorrelation of the two methods and by comparison with published empirical data. The significant correlation between the two estimates of P/B was expected, as the two predictors (longevity and size-at-maturity) were themselves correlated, as discussed above. Both methods use different predictors to estimate the same parameter. The second method (P/B_W) is favoured because the predictor population trait (size-at-maturity or maximum size) is often available from population studies of age and growth and because the empirical relationship between animal size and P/B is well founded in the literature (Banse and Mosher 1980; Peters 1983).

Assuming that fish weight is potentially the most useful predictor of P/B, the allometric coefficient that relates P/B to size is important. The $\log P/B_Z$ scaled with log fish weight with a slope of –0.19, which was similar to the physiological scaling (–0.25) discussed earlier. For their whole data set, Downing and Plante (1993) found that P/B and weight were related with an exponent of –0.22 (n = 100 populations, major axis regression). However, for the subset of data that were used for validation in this study, P/B scaled with fish size as $W^{-0.35}$, after adjustment for biomass (n = 24 populations). This greater coefficient is consistent with the allometric coefficient used to calculate P/B_W in this study. The wide confidence interval in the regressions of P/B on weight is a reminder that body size is an inexact predictor of P/B (Banse and Mosher 1980; Randall et al. 1995), and predic-

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tions of specific production from body mass are approximations. The latter slope of –0.35 is similar to the within fish group (population production) coefficients discussed by Dickie et al. (1987) and Boudreau and Dickie (1989). Until further validation with field data is possible, the coefficient of –0.35 will be used to estimate P/B_W ($= aW^{-0.35}$).

The P/B_W consistently underestimated the observed field P/B ratios. This suggests that the proportionality coefficient (a) of the allometric equation could be adjusted upwards. There are two reasons why this may be the case. First, Randall et al. (1995) used average fish weight rather than W_{mat}. Average fish weight in populations of actively growing fish would be less than maturity weight, and average P/B based on maturity weight would be underestimated accordingly. Second, Randall et al.'s (1995) equation relating fish weight to P/B was based on community measures of fish production. Downing and Plante (1993) showed that community production, adjusted for fish biomass, was marginally lower than population production. Again, this would lead to an underestimate of P/B. The average ratio of observed to predicted production, using the unadjusted proportionality coefficient, was 2.04 (excluding yellow perch); P/B ratios adjusted by a factor of 2 (coefficient of 2.64 rather than 1.32) produced a reasonably good fit with the P/B ratios observed from the field studies. Use of the published data for calibration would suggest that P/B from species W_{mat} data can be estimated as $2.64W^{-0.35}$. Given the above uncertainties, both the proportionality coefficient and exponent of this equation may be modified in future.

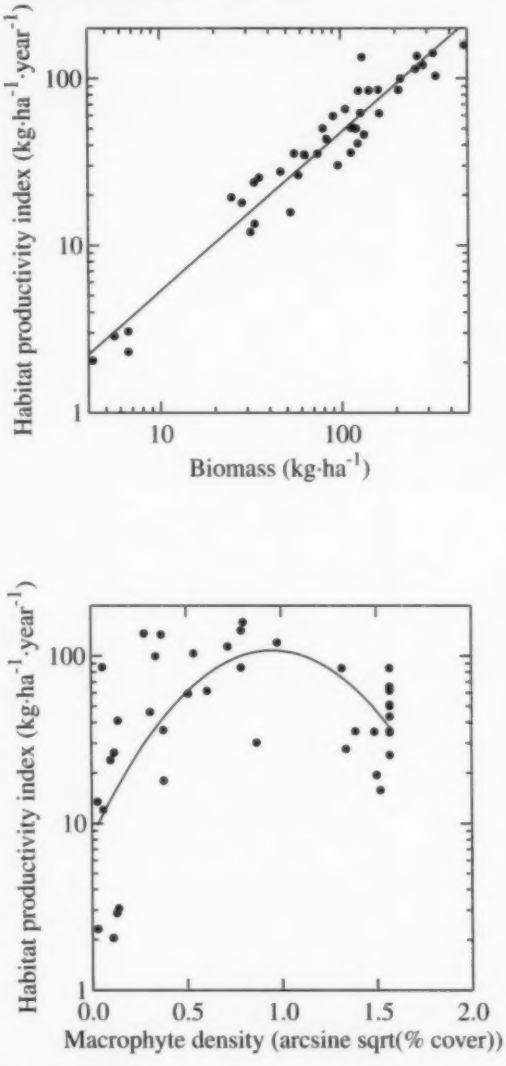
P/B values estimated for all 79 species of fish using this equation are reported in Table 1. Further validation of these ratios is needed, particularly for species that mature at a large size and live for many years, since our data set was based primarily on short-lived species. P/B coefficients in Table 1 are provisional. Fishes inhabiting rivers would have higher P/B ratios for any given biomass; P/B coefficients may have to be adjusted upwards by 1.5 for river habitats (Randall et al. 1995). Populations of young fish (e.g., juvenile anadromous salmonids) would have higher P/B ratios than indicated in Table 1. Additional adjustments to the P/B ratios could be made to account for differences in water nutrient levels (total phosphorus concentrations) or water temperature (Downing and Plante 1993). Although P/B coefficients for freshwater species of fishes are considered in this study, the same principle and methodology for estimating P/B would apply to marine fishes, but the coefficients would have to be validated by comparison with estimates of fish production in marine habitats.

Application

A first-order habitat productivity index (HPI), a proposed measure of habitat suitability and productive capacity, can be calculated as the product of average fish biomass and the species P/B coefficient (HPI = $B \times P/B$). This fish production index assumes that fish production is correlated with average fish biomass and that biomass is linked to habitat productivity, and the P/B coefficient is a further refinement to adjust for the effects of fish size on population production.

As an example, HPI values were calculated for a number of samples from boat electrofishing transects in nearshore areas of Lake Erie (n = 30) and Lake Ontario (n = 33). The fish samples were collected by boat electrofishing at 100-m line transects in shallow waters (1.5 m depth). The habitats surveyed included coastal wetlands (with submerged macrophytes), harbour breakwalls, and exposed shorelines adjacent to the harbours (R.G. Randall, personal observation). Sixty-three transects were surveyed on three occasions at monthly intervals during the summer of 1994. For calculation of biomass density, we assumed that the effective width

Fig. 6. Top panel: correlation between average fish biomass from electrofishing samples and the HPI. Fish catches were from samples collected by boat electrofishing at 100-m line transects in nearshore habitats of Lakes Erie and Ontario (R.G. Randall, personal observation). HPI was the product of average biomass and species P/B (per year) ratios, summed for all species captured at the transect. Axes are on a $\log_{10}$ scale. Bottom panel: relationship between the calculated HPI and macrophyte density (% bottom cover, arcsine square root transformed) at the transects. Only transects where macrophytes were present were included (n = 43).



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of each transect was 10 m, and the average catch efficiency was 0.3 (Randall et al. 1993). Average biomass (kilograms per hectare) for the three visits was calculated for each transect, by species. HPI was calculated as the product of the average biomass and the species *P/B* ratios, summed for all species captured at each transect. Transects that included an average of at least five fish were included in the analysis ($n = 54$).

HPI was highly correlated ($R^2 = 0.93$) with biomass, as expected by calculation, but it varied for any specific level of biomass because of differences in fish size (Fig. 6). For a transect where the total catch in biomass was 30 kg·ha^{-1}, for example, the 95% confidence interval for HPI ranged between 10.7 and 29.5 kg·ha^{-1}·year^{-1}, a factor of about 3. Both biomass and fish size were significant predictors of HPI ($P < 0.05$), confirming that HPI was related to fish size in the field catch data. Preliminary investigation showed that the residuals from the regression of HPI versus biomass were weakly correlated with macrophyte abundance ($r = 0.27$, $P = 0.05$), indicating that HPI was related to habitat cover after adjustment for biomass. Macrophyte abundance affects fish assemblage structure and possibly habitat productivity (Randall et al. 1996) (Fig. 6, bottom panel). These observations suggest that although HPI provided a similar measure of habitat capacity as biomass, it also accounted for fish size structure. Areas with similar biomass but with smaller fish size had a higher HPI than areas with larger fish. HPI is a more direct fish assemblage measure of habitat productive capacity than biomass alone, although this hypothesis has yet to be tested in an area where fish production has been estimated in the littoral zone of a lake. These preliminary results suggest that further investigation and testing of the HPI could be worthwhile.

A more sensitive fish metric of production would be to adjust *P/B* for the size of the fish in the catches rather than using a constant *P/B* coefficient for each species. This refinement will require investigation of the relationship between fish size and *P/B* within and among cohorts of different populations and species, as has been initiated by Boudreau and Dickie (1989). HPI as an index of habitat productive capacity requires further validation by comparison with biomass spectra (Boudreau and Dickie 1992) and field estimates of population and community production.

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Appendix 1. Length–weight regression coefficients for fish species collected as part of the Productive Capacity fish database. Species codes are defined in Table 1.

Species code	n	L_{min}	L_{max}	W_{min}	W_{max}	b	SE_b	a	SE_a	R^2	$SE_{estimate}$
F0051	75	267	762	175	6 000	3.04	0.08	–5.10	0.21	0.95	0.07
F0061	9873	15	192	1	71	2.75	0.01	–4.44	0.02	0.93	0.08
F0063	679	31	455	1	1 700	3.01	0.01	–4.82	0.03	0.99	0.10
F0075	101	73	850	3	8 100	2.93	0.11	–4.82	0.21	0.89	0.12
F0076	32	65	565	3	2 440	3.00	0.04	–4.93	0.08	0.99	0.05
F0078	38	73	617	11	3 180	2.84	0.12	–4.40	0.31	0.94	0.19
F0121	75	47	185	1	29	2.90	0.16	–5.00	0.32	0.82	0.20
F0131	269	44	955	1	5 540	3.00	0.02	–5.19	0.06	0.99	0.07
F0133	47	99	277	6	144	3.08	0.08	–5.37	0.17	0.97	0.06
F0161	25	243	533	220	2 400	3.32	0.13	–5.62	0.33	0.97	0.05
F0163	1036	65	513	3	1 594	2.99	0.01	–4.86	0.02	0.99	0.05
F0164	15	66	237	2	231	3.27	0.17	–5.37	0.36	0.97	0.13
F0171	59	163	541	56	2 800	3.07	0.08	–5.01	0.20	0.96	0.05
F0173	26	308	620	408	3 610	3.06	0.11	–5.00	0.30	0.97	0.03
F0181	76	121	563	46	3 000	2.25	0.20	–2.71	0.49	0.64	0.15
F0186	1807	115	910	32	15 360	2.95	0.02	–4.56	0.06	0.91	0.08
F0194	394	36	164	1	62	3.07	0.05	–5.05	0.09	0.91	0.14
F0196	2506	35	154	1	29	2.95	0.03	–4.96	0.06	0.79	0.13
F0199	214	33	84	1	6	2.49	0.16	–4.05	0.27	0.53	0.13
F0201	1054	32	120	1	24	2.96	0.04	–4.85	0.08	0.82	0.16
F0208	88	43	83	1	10	3.32	0.22	–5.55	0.39	0.72	0.13
F0233	3928	70	430	5	1 066	2.97	0.01	–4.77	0.03	0.92	0.07
F0251	143	53	1030	90	3 260	1.87	0.13	–2.39	0.37	0.59	0.22
F0291	16	67	125	2	28	4.56	0.36	–8.01	0.71	0.92	0.09
F0301	2730	38	330	1	680	3.13	0.01	–5.05	0.02	0.97	0.07
F0302	71	80	335	8	710	2.60	0.13	–3.80	0.31	0.85	0.12
F0311	931	42	268	1	450	2.99	0.02	–4.62	0.03	0.98	0.07
F0313	5154	22	256	1	225	3.12	0.01	–4.85	0.01	0.98	0.08
F0314	529	8	196	1	222	3.02	0.03	–4.67	0.06	0.95	0.16
F0316	527	28	432	1	1 346	2.92	0.02	–4.61	0.04	0.98	0.09
F0317	1859	30	457	1	1 830	3.03	0.01	–4.87	0.02	0.99	0.09
F0319	362	29	318	1	585	2.96	0.02	–4.70	0.04	0.98	0.11
F0331	4797	37	350	1	560	2.96	0.01	–4.77	0.02	0.95	0.09
F0334	290	70	682	4	5 300	3.02	0.03	–5.01	0.06	0.98	0.13
F0342	1479	52	142	1	33	2.82	0.03	–4.66	0.07	0.82	0.13
F0371	181	138	570	25	3 980	3.32	0.06	–5.74	0.15	0.95	0.07

Note: Coefficients are for the regression log weight = log a + b(log length), where length is FL (mm) and weight is wet weight (g).

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Appendix 2. Length–weight coefficients for five species of fish, as obtained from the literature.

Species	Code	*n*	*a*	*b*	Units	Reference
Fathead minnow	F0209	387	–3.26	2.28	log weight (g); log SL (mm)	Chadwick 1976*
Pearl dace	F0214	405	–3.79	2.53	log weight (g); log SL (mm)	Chadwick 1976
Creek chub	F0212	235	–3.85	2.57	log weight (g); log SL (mm)	Chadwick 1976
Brook trout	F0080		–5.1142	3.06	log weight (g); log FL (mm)	Carlander 1969† (mean of 6 populations)
Lake whitefish	F0091		–5.1483	3.11	log weight (g); log FL (mm)	Carlander 1969 (mean of 8 populations)

*Carlander, K.D. 1969. Handbook of freshwater fishery biology. Vol. 1. Iowa State University Press, Ames, Iowa.
†Chadwick, E.M.P. 1976. Ecological fish production in a small Precambrian shield lake. Environ. Biol. Fishes, 1: 13–60.

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Physiological status of coho salmon (*Oncorhynchus kisutch*) captured in commercial nonretention fisheries

A.P. Farrell, P. Gallaugher, C. Clarke, N. DeLury, H. Kreiberg, W. Parkhouse, and R. Routledge

Abstract: The physical, hematological, and metabolic condition of 303 adult coho salmon (*Oncorhynchus kisutch*) was examined following capture with three commercial fishing gear types (seine, troll, and gill net) and a variety of methods. All fish arrived onboard in a state of severe metabolic exhaustion, and physiological differences among gear types and fishing methods were few and relatively small. Fish showed less physiological disruption with a brailing versus a ramping method of seine fishing and with a 30-min versus a 60-min net soak time for gillnet fishing. The visual ratings of physical condition (nonbleeding, vigorous, and lethargic) correlated significantly with hematocrit, plasma osmolality, plasma lactate, and plasma sodium. Fish placed in recovery boxes for 30–60 min onboard fishing vessels did not show the expected metabolic recovery; only plasma potassium recovered significantly. However, plasma lactate levels declined significantly for 125 fish placed in a net pen for 24 h, suggesting that metabolic recovery was possible after commercial capture. Because of a concern that the current recovery box design does not effect optimum recovery, we recommend that future experiments test a better-designed recovery box that orients fish into flowing water.

Résumé : Nous avons examiné la condition physique, hématologique et métabolique de 303 cohos (*Oncorhynchus kisutch*) adultes après leur capture dans trois types d'engins de pêche commerciale (senne, lignes et filet maillant). Tous les poissons arrivaient à bord dans un état de grave épuisement métabolique, et les différences physiologiques étaient peu nombreuses et relativement faibles d'un type d'engin et d'une méthode de pêche à l'autre. Les poissons présentaient moins de perturbations physiologiques dans le cas du salabardage qu'avec le hissage sur la rampe dans la pêche à la senne, et avec un temps de mouillage de 30 min qu'avec 60 min dans la pêche aux filets maillants. Les évaluations visuelles de la condition physique (absence de saignement, poisson vigoureux ou léthargique) étaient significativement corrélées à l'hématocrite, à l'osmolalité plasmique et aux concentrations de lactate et de sodium dans le plasma. Les poissons placés dans des viviers de récupération pendant 30–60 min à bord des bateaux de pêche se présentaient pas le rétablissement métabolique prévu, car seul le potassium plasmique remontait de façon notable. Toutefois, les concentrations plasmiques de lactate baissaient de façon significative chez 125 poissons placés dans un parc de filet pendant 24 h, ce qui permet de penser que la récupération métabolique est possible après la capture commerciale. Il semble que la conception actuelle des viviers de récupération ne soit pas optimale, aussi recommandons-nous que des expériences futures mettent à l'essai un vivier mieux conçu qui oriente les poissons dans un courant d'eau.

[Traduit par la Rédaction]

Introduction

In response to the grave concerns about the health of some coho salmon (*Oncorhynchus kisutch*) stocks, the Minister of Fisheries and Oceans announced a coastwide policy of selective fisheries for the 1998 commercial salmon fishing season. The selective fisheries policy made it mandatory for all British Columbia commercial salmon fishing vessels to carry and use recovery boxes ("blue boxes") to recover nontarget species onboard the vessel prior to release. However, the concept of live release of nontarget species of salmon after capture (i.e., nonretention fishing) is relatively new within the commercial fishing industry in British Columbia, even though both gear and fishing technique are being examined with the intention of reducing the mortality of nontarget species (Weigold and Cook 1995; J.O. Thomas and Associates, Ltd. 1997).

Furthermore, to our knowledge, the benefits of these blue boxes have never been appropriately tested. Previously, recovery boxes (which are based on a 50- to 100-L plastic tub with a lid and a running water supply) were assessed as being "very effective in increasing survival of non-target spe-

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A.P. Farrell.[1] Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.
P. Gallaugher. Department of Continuing Studies in Science, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.
C. Clarke and H. Kreiberg. Fisheries and Oceans Canada, Pacific Biological Station, Nanaimo, BC V9R 5K6, Canada.
N. DeLury and R. Routledge. Department of Mathematics and Statistics, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.
W. Parkhouse. Department of Kinesiology, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

[1]Author to whom all correspondence should be addressed.
e-mail: farrell@sfu.ca

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cies in some cases" (Blewett and Taylor 1999). Yet, earlier studies had cast doubt on whether "recovery" did indeed occur using such boxes; Parker et al. (1959) and later Ellis (1964) had concluded that live tank holding of salmon caused elevated lactate levels in troll-caught coho and chinook salmon (*Oncorhynchus tshawytscha*).

The purpose of the present study, therefore, was to make an assessment of the benefits of the recovery box for commercially caught coho salmon based on a broad range of physiological measurements. However, the physiological changes associated with the commercial capture of wild salmon are poorly understood because of limited studies. Muscular exhaustion was indicated by elevated muscle lactate levels in troll-caught wild Pacific coho and chinook salmon (Parker et al. 1959; Parker and Black 1959), while stress and scale loss were linked to mortality of adult sockeye salmon (*Oncorhynchus nerka*) caught in gill nets (Thompson et al. 1971). Reports on fish mortality rates subsequent to capture with commercial fishing and sportfishing methods also exist (sportfishing: e.g., Vincent-Lang et al. 1993; Cox-Rogers 1998; commercial fishing: e.g., Candy et al. 1996; see also Chopin and Arimoto 1995).

In contrast, there is extensive documentation of the physiological changes occurring when hatchery-raised salmonids recover from exhaustive exercise (reviewed by Milligan 1996) and in wild Atlantic salmon (*Salmo salar*) following sport angling and induced exhaustion (Booth et al. 1995; Brobbel et al. 1996; Wilkie et al. 1996, 1997). Exhaustive exercise typically increases blood lactate, muscle lactate, plasma ions, hematocrit (Hct), and stress hormones (e.g., catecholamines and cortisol), while muscle phosphocreatine (PCr), adenosine triphosphate (ATP), and glycogen decrease. These variables then recover over periods of minutes (e.g., PCr) to many hours (e.g., glycogen). The presumption is that commercially caught wild salmon can recover in a similar manner, but this presumption needs testing if there is to be a sound scientific foundation for effective nonretention commercial salmon fishing. To test this presumption, we predicted that some of the physiological indicators would show recovery after 30 or 60 min in the recovery box and after 24 h in a net pen when compared with fish that were sampled immediately upon capture (i.e., zero recovery time). In addition, comparisons were made among and within the gear types for seine, gillnet, and troll fishing.

Material and methods

Overview

This study was conducted in Barkley Sound, B.C., in September 1998. Representatives of the commercial seine, gillnet, and troll salmon fishing fleets, in collaboration with Fisheries and Oceans Canada, targeted a healthy stock of Alberni Inlet coho salmon. We assessed the effectiveness of the recovery box onboard commercial fishing vessels by measuring muscle and plasma indicators that quantify physiological exhaustion. Recovery boxes used on seine vessels were a plastic commercial "half-tote," 110 × 57 × 67 cm (external length, width, and height), supplied by a dedicated auxiliary 3.75-cm pump drawing from a 3- to 10-m intake line hung over the vessel's side. Recovery boxes used on gillnet and troll vessels were smaller, approximately 90 × 40 × 40 cm (length, width, and height), and water supply arrangements varied. Average flow rates varied from 0 to 25 $L \cdot min^{-1}$ and seawater temperatures ranged from 12 to 19°C depending on the intake depth. Modifications to standard fishing methods were as follows. For gillnet fishing, net soak times of 30 and 60 min were compared. For seine fishing, a traditional ramping method and seine was compared with a dry brailing method using either a regular seine or a seine with a modified bunt. For the troll fishing, fish were released directly off the hook at the water line without air exposure. Although several boats of each gear type were used in the experiment and a total of 303 coho salmon were sampled, logistics often dictated that only one boat was used to test any given fishing method. The variables that we measured included muscle glycogen, muscle lactate, muscle glucose, muscle PCr, plasma lactate, and plasma glucose (all indicators of fatigue), Hct (an indicator of bleeding, air exposure, and stress), and plasma osmolality and ion (Na^+, K^+, and Cl^-) concentrations (indicators of ionic and osmotic shock). An additional experiment involved a further 125 coho salmon that were transferred to a 6-m^3 net pen immediately after capture. Delayed mortality and physiological status of the plasma were assessed after a 24-h observation period. Unfortunately, a problem with the anticoagulant precluded reliable use of Hct and plasma ion data for the net pen samples and only plasma lactate and glucose values are reported here.

Experimental protocol

Directly upon landing, fish condition was visually rated by an observer (Fisheries and Oceans Canada ratings: 1 = lively and no bleeding, 2 = lively but bleeding, 3 = lethargic and no bleeding, 4 = lethargic but bleeding, and 5 = no sign of ventilatory movements). Few fish were bleeding and so the majority of fish used in the experiment were visually rated as either 1 or 3. Fork length of the fish ranged from 53.5 to 80.0 cm. The fish were randomly assigned to a 0- (zero recovery), 30-, or 60 min recovery period and tissue and blood samples were taken at these three sample times. Prior to sampling, fish were stunned by a sharp blow to the skull, immediately placed in a V-shaped trough, and a muscle sample (approximately 0.5 × 1.0 × 1.0 cm) excised from below and slightly to the anterior of the dorsal fin in <45 s. The muscle biopsy was instantly frozen between metal tongs precooled on dry ice. The frozen tissue was placed in tinfoil and stored on dry ice. Directly following removal of the muscle tissue, blood was drawn by caudal puncture into a 3-mL heparinized vacutainer and two heparinized hematocrit tubes were filled immediately from the blood sample. Whole blood and hematocrit tubes were stored (<20 min) on crushed ice before they were centrifuged onboard the vessel. Plasma was aliquoted into 1-mL Eppendorf tubes that were then frozen on dry ice. The muscle and plasma samples were subsequently stored at –80°C until analysis in the laboratory. Fish that were sampled from the net pen were quietly gathered and dipnetted into an anesthetic bath (clove oil, 50–75 $mg \cdot L^{-1}$), where blood was sampled as above without killing the fish.

Analytical techniques

Blood and muscle tissues

Plasma lactate and glucose concentrations were measured using a YSI 2300 StatPlus lactate/glucose analyzer (Yellow Springs Instruments). Plasma samples were thawed immediately before use, vortexed for 30 s, spun in a centrifuge for 2 min at 2000 rpm, and then aspirated into the analyzer. The analyzer was set to automatically calibrate after every five measurements (precision for lactate = 0.2 $mmol \cdot L^{-1}$ and for glucose = 0.1 $mmol \cdot L^{-1}$). Duplicate samples were within 2% of each other. Muscle tissue samples were powdered under liquid nitrogen using a precooled mortar and pestle. Approximately 500 mg of powdered tissue was added to a precooled, preweighed vial containing 1 mL of ice-cold 0.6 N perchloric acid. Each vial was reweighed and made to a final dilution of seven volumes (volume/weight). The perchloric acid

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Table 1. Plasma and muscle variables in mature coho salmon that were caught by commercial fishing vessels: a comparison between holding times in recovery boxes onboard vessels for all gears combined.

	Recovery time[a]			Level of significance[b]		
Response variable	0 min	30 min	60 min	0 vs. 30 min	0 vs. 60 min	30 vs. 60 min
Plasma Cl^- (mequiv.·L^{-1})	143.6	151.9	152.3	***	***	ns
Plasma Na^+ (mequiv.·L^{-1})	171.4	185.4	190.9	***	***	***
Plasma K^+ (mequiv.·L^{-1})	4.0	3.5	3.7	*	ns	ns
Osmolality (mosmol)	350.5	380.4	390.7	***	***	**
Hct (%)	48.9	50.1	49.8	ns	ns	ns
Plasma lactate (mmol·L^{-1})	11.5	18.0	23.2	***	***	***
Plasma glucose (mmol·kg^{-1})	6.16	8.05	8.16	***	***	ns
Muscle lactate (mmol·kg^{-1})	54.7	53.9	52.2	ns	ns	ns
Muscle glucose (mmol·kg^{-1})	2.35	2.48	2.49	ns	ns	ns
Muscle glycogen (mmol·kg^{-1})	2.04	2.02	1.18	ns	ns	ns
Muscle PCr (mmol·kg^{-1})	2.36	1.14	2.53	ns	ns	ns

[a]Least squares means. *N* values varied as follows: 103–109 and 58–65 for time zero plasma and tissue variables, respectively, 103–108 and 50–60 for time 30 min plasma and tissue variables, respectively, and 94–97 and 52–55 for time 60 min plasma and tissue variables, respectively.

[b]Two-way analysis of variance with interactions ($\alpha = 0.05$ with additional protection for multiple comparisons): ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table 2. Plasma and muscle variables in mature coho salmon that were caught by commercial fishing vessels: a comparison between commercial fishing gear types at time of capture (zero recovery time).

	Gear type[a]			Level of significance[b]		
Response variable	Gill net	Seine	Troll	Gill net vs. seine	Gill net vs. troll	Seine vs. troll
Plasma Cl^- (mequiv.·L^{-1})	146.9	141.2	144.4	**	ns	ns
Plasma Na^+ (mequiv.·L^{-1})	179.6	168.4	164.5	***	***	ns
Plasma K^+ (mequiv.·L^{-1})	4.1	3.7	4.3	ns	ns	ns
Osmolality (mosmol)	373.9	343.8	324.9	***	***	ns
Hct (%)	51.9	48.5	44.6	ns	ns	ns
Plasma lactate (mmol·L^{-1})	16.9	9.1	7.7	***	***	ns
Plasma glucose (mmol·kg^{-1})	6.9	5.9	5.7	ns	ns	ns
Muscle lactate (mmol·kg^{-1})	50.2	58.7	52.3	*	ns	ns
Muscle glucose (mmol·kg^{-1})	2.24	2.47	2.23	ns	ns	ns
Muscle glycogen (mmol·kg^{-1})	1.17	3.00	1.25	ns	ns	ns
Muscle PCr (mmol·kg^{-1})	2.20	1.86	4.32	ns	ns	ns

[a]Least squares means for holding time equal to zero. *N* values varied as follows: 146–172 and 82–91 for seine plasma and tissue variables, respectively, 113–119 and 58–65 for gillnet plasma and tissue variables, respectively, and 22–23 and 20–22 for troll plasma and tissue variables, respectively.

[b]Two-way analysis of variance with interactions using the data from all holding times ($\alpha = 0.05$ with additional protection for multiple comparisons): ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

extracts were homogenized on ice for 2 × 15 s at maximum speed with an Ultraturex tissue homogenizer. An aliquot was immediately frozen in liquid nitrogen for determination of glycogen. The remaining perchloric acid extract was then centrifuged for 2 min at 13 000 rpm in a microcentrifuge. A known volume of supernatant was immediately removed and neutralized with tris(hydroxymethyl)-aminomethane. The neutralized extracts were stored at –80°C until analysis. Glycogen was digested with amyloglucosidase (Bergmeyer 1983) and glucosyl units determined on the YSI 2300 StatPlus lactate/glucose analyzer. Final glycogen values were determined after subtracting muscle glucose values. Muscle glucose and lactate values were determined on the extracts with the analyzer as above. Muscle PCr concentrations were determined enzymatically by following the production of nicotinamide adenine dinucleotide phosphate at 340 nm (Bergmeyer 1983).

Plasma ions and osmolality

Plasma samples were thawed, vortexed, and centrifuged for 5 min immediately before analysis. Plasma Cl^- concentrations ($[Cl^-]$) were measured in duplicate using a model 4425000 Haake Buchler digital chloridometer. The measurements were repeated if there was disagreement between duplicates >2.5 mequiv.·L^{-1}. The chloridometer was checked against a Cl^- standard (100 mequiv. Cl^-·L^{-1}) before and during the process (approximately every 10 duplicates). Concentrations of plasma Na^+ ($[Na^+]$) and K^+ ($[K^+]$) were measured using a model 510 Turner flame photometer. Plasma aliquots (5 µL) were diluted 1:200 with a prepared 15 mequiv. lithium·L^{-1} diluent for analysis. The machine was calibrated prior to use and checked against a standard approximately every six samples. Measurements were repeated if the disagreement between duplicates was >2%. Osmolality was measured in duplicate on 10-µL samples using a model 5500 Wescor vapour pressure. The thermocouple heads were periodically cleaned in order to maintain consistency. Measurements were repeated if the disagreement between duplicates was >3%.

Statistical analysis

Of primary interest were the existence of (*i*) differences between

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Fig. 1. (A) Plasma lactate and (B) plasma glucose concentrations measured in mature coho salmon caught by three commercial gear types (seine, open bars; troll, solid bars; gill net, hatched bars). Blood samples were taken at the time of capture, after 30 and 60 min in recovery boxes, and after 24 h of recovery in a net pen. Mean values are presented and the SEM is indicated by the vertical bar. At capture, 15–21 fish were sampled for each seine vessel, 16–22 samples for each gillnet vessel, and four to nine samples for each troll vessel. For the net pen, *N* values were 29–40 (seine), 4–13 (gill net), and 17 (troll). Statistically significant differences between 60 min and 24 h recovery time for each gear type ($p < 0.05$) are indicated by dissimilar letters.

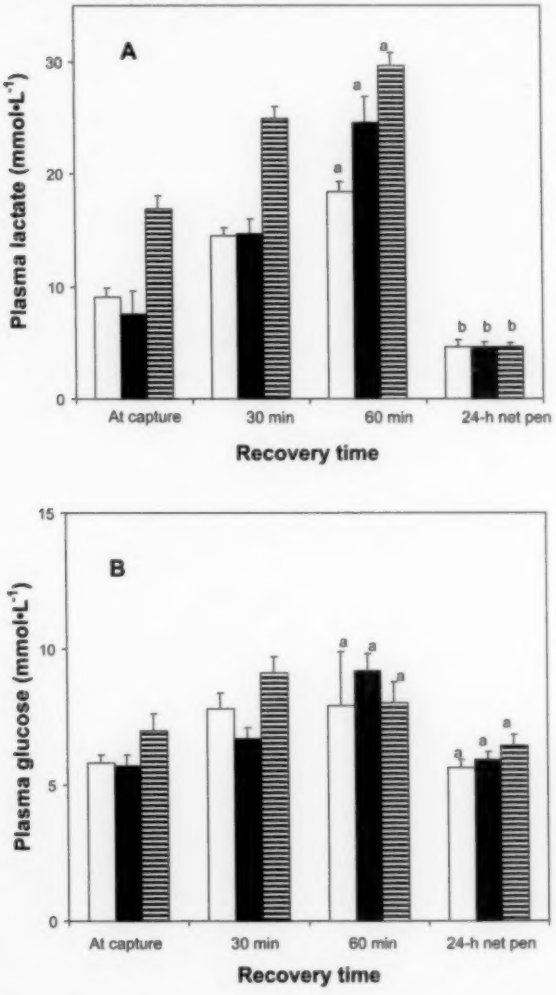
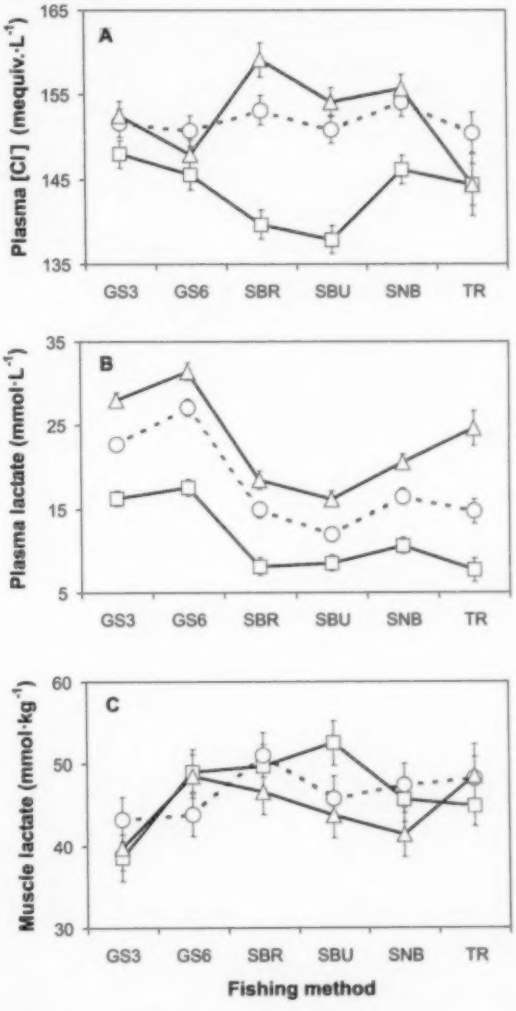


Fig. 2. (A) Plasma Cl^-, (B) plasma lactate, and (C) muscle lactate concentrations in mature coho salmon after zero (squares), 30 (triangles), or 60 min (circles) of recovery. Statistically significant interactions between fishing gear methods and recovery time are illustrated by inconsistent differences over time and among fishing methods. In the case of plasma Cl^- and plasma lactate, but not muscle lactate, these interactions were evident from simply a change in the pattern among fishing methods with recovery time. GS3 and GS6 refer to gillnet soak times of 30 min and 60 min, respectively, SR refers to a traditional ramping method with seine, while SBR refers to a dry brailing method using either a regular seine, SBU refers to a dry brailing method with a seine with a modified bunt, and TR refers to troll fishing. Mean values are presented and the SEM is indicated by the vertical bar.



fishing methods in stress indicators when the fish were brought onboard, (*ii*) consistent changes (across all gear types and fishing methods) in the stress indicators over time spent in the recovery boxes, and (*iii*) differences between fishing methods in these changes over time. Tests for these were done through two-factor analyses of variance with fixed effects for the first two items above, interactions to address the third item, and a 5% significance level. The analysis of variance model was specially coded (using ordinal variables in JMP, SAS Institute Inc., Cary, N.C.) so as to direct the test for differences between fishing methods at zero recovery time. Where possible, we tested for boat-to-boat differences and found no significance. These were therefore not included in the main analysis of variance models. All conclusions are contingent on the absence of vessel effects. Results were also tested for

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Table 3. Plasma and muscle variables in mature coho salmon that were caught by commercial fishing vessels: a comparison of gear types among fishing methods.

Method	Recovery (min)		Plasma							Muscle tissue			
			Hct (%)	Cl^- (mequiv.·L^{-1})	Na^+ (mequiv.·L^{-1})	K^+ (mequiv.·L^{-1})	Osmolality (mosmal)	Lactate (mmol·L^{-1})	Glucose (mmol·L^{-1})	Lactate (mmol·kg^{-1})	Glucose (mmol·kg^{-1})	Glycogen (mmol·kg^{-1})	PCr (mmol·kg^{-1})
Seine gear													
Brailing	0	Mean	45.8	139.7 *a*	165.7 *a*	4.2	340.0 *a*	8.1 *a*	5.0 *a*	57.2 *a*	2.37	2.41	1.24
		SEM	2.1	1.5	2.7	0.3	3.9	0.7	0.3	3.7	0.16	1.00	0.79
		N	17	18	18	18	18	17	17	10	10	9	10
	30	Mean	48.9	153.2	187.9	2.7	379.1	14.9	8.1	59.5	3.03	1.72	1.64
		SEM	1.7	1.4	1.9	0.2	3.2	0.7	0.5	2.0	0.34	0.81	0.57
		N	18	18	18	18	18	18	18	9	9	7	9
	60	Mean	47.2	159.1	193.2	3.0	383.9	18.4	7.9	55.7	2.48	1.15	0.38
		SEM	1.7	3.0	3.1	0.2	4.1	1.1	0.8	2.5	0.12	0.51	0.14
		N	16	14	14	14	14	15	15	10	10	10	10
Modified bunt	0	Mean	50.3	137.9 *b*	165.8 *a*	3.5	341.4 *a*	8.5 *a*	6.2 *ab*	62.5 *a*	2.66	4.24	2.09
		SEM	1.7	1.0	1.5	0.4	4.0	1.0	0.6	2.7	0.17	1.19	0.56
		N	19	21	21	21	21	21	21	11	11	11	11
	30	Mean	51.6	150.9	178.3	3.9	367.5	12.0	7.1	54.9	2.56	1.52	2.93
		SEM	1.3	2.0	1.8	0.3	3.5	0.6	0.3	2.4	0.12	0.50	1.29
		N	20	20	20	20	20	20	20	10	10	10	8
	60	Mean	52.3	154.1	185.7	3.4	390.3	16.2	7.6	51.7	2.16	1.05	3.30
		SEM	1.5	1.7	1.2	0.4	5.2	0.8	0.5	3.2	0.13	0.37	1.46
		N	20	19	19	19	19	20	20	10	10	8	10
Ramping	0	Mean	49.0	146.2 *c*	173.8 *ab*	3.5	350.1 *a*	10.6 *a*	6.2 *ab*	52.2 *a*	2.24	2.45	2.13
		SEM	2.1	1.9	1.6	0.3	4.8	0.6	0.3	2.4	0.09	0.80	0.51
		N	19	19	19	19	19	20	20	10	10	10	10
	30	Mean	52.2	154.2	187.3	2.1	383.2	16.5	8.2	54.9	2.46	3.59	1.37
		SEM	1.5	2.1	1.6	0.2	7.7	0.7	0.4	1.6	0.10	1.00	0.59
		N	20	20	20	20	20	20	20	11	11	11	11
	60	Mean	48.9	155.7	192.0	2.4	392.2	20.6	8.2	49.1	2.56	1.67	3.30
		SEM	2.3	1.8	1.1	0.4	4.7	0.9	0.6	2.0	0.13	0.40	0.96
		N	20	20	20	20	20	20	20	11	11	11	11
Gillnet gear													
30-min soak time	0	Mean	50.5	148.0 *c*	182.0 *b*	3.9	379.8 *b*	16.2 *b*	6.5 *ab*	43.6 *ab*	2.08	1.21	2.43
		SEM	1.7	1.2	2.0	0.3	3.6	0.8	0.4	2.3	0.07	0.42	1.50
		N	22	21	22	22	22	22	22	12	12	11	12
	30	Mean	51.1	151.7	193.0	3.8	396.6	22.7	9.4	50.0	2.41	2.77	0.53
		SEM	0.9	1.1	1.6	0.3	3.1	0.9	0.5	2.2	0.10	0.83	0.26
		N	21	20	20	20	20	21	21	11	11	11	11
	60	Mean	49.3	152.6	197.1	3.6	405.1	28.0	8.6	46.4	2.73	2.24	4.27
		SEM	1.5	2.0	1.8	0.3	3.9	0.8	0.7	4.2	0.11	0.97	1.54
		N	21	21	21	21	21	21	21	10	10	10	10

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60-min soak time	0	Mean	53.4	145.6 *a*	176.7 *bc*	4.3	367.0 *ab*	17.5 *b*	7.4 *b*	56.8 *a*	2.40	1.13	1.93
		SEM	1.9	1.4	3.3	0.3	7.6	1.7	0.8	2.4	0.08	0.40	0.85
		N	18	18	18	18	18	18	18	11	11	11	9
	30	Mean	50.6	150.8	190.5	3.9	404.0	27.1	8.8	49.4	2.13	1.49	1.04
		SEM	2.4	1.1	1.6	0.5	3.5	1.3	0.7	2.4	0.18	1.00	0.68
		N	19	17	17	17	17	18	18	11	11	11	7
	60	Mean	50.5	147.9	194.5	5.6	403.9	31.4	7.5	54.0	2.40	0.02	2.63
		SEM	1.6	1.8	1.6	0.5	4.0	1.4	0.8	2.6	0.16	0.02	0.57
		N	15	16	16	16	16	16	16	10	10	10	10
Troll gear	0	Mean	44.6	144.4 *a*	164.5 *c*	4.3	324.9 *a*	7.6 *a*	5.7 *ab*	52.3 *a*	2.23	1.25	4.32
		SEM	1.7	2.9	4.0	0.2	9.3	2.0	0.3	3.4	0.13	0.44	1.32
		N	9	9	9	9	10	9	9	10	10	10	10
	30	Mean	46.0	150.5	175.4	4.3	351.8	14.7	6.7	56.2	2.42	1.45	0.06
		SEM	4.3	3.2	2.5	0.2	6.1	1.3	0.6	3.8	0.13	1.00	0.03
		N	9	9	9	9	9	9	9	8	8	6	8
	60	Mean	49.5	144.4	182.9	4.1	368.8	24.6	9.2	56.6	2.57	0.86	2.37
		SEM	2.6	3.4	2.9	0.4	14.0	2.3	2.0	6.7	0.23	0.68	1.24
		N	4	4	4	4	4	4	4	4	4	4	3

Note: Statistical differences ($p < 0.05$, two-way analysis of variance with interactions) are indicated only between time zero values by a dissimilar letter.

physiological differences between visual rankings (1 and 3) of fish condition using a single-factor analysis of variance, again with a 5% significance level. All analyses were followed by multiple comparison tests (with the experiment-wise error rate fixed at 5%) to determine which means were significantly different. Least-squares means were used to adjust for imbalances in the design.

Results

Physiological changes with time in the recovery box

The data for the overall effect of recovery time on the physiological variables are summarized in Table 1. The mean Hct value (48.9% immediately after capture) did not change significantly after either 30 or 60 min of recovery (Table 1).

Plasma ions changed significantly during the recovery period. Both plasma [Na^+] (171.4 mequiv.·L^{-1} immediately after capture) and plasma osmolality (350.5 mosmol immediately after capture) increased significantly after 30 min and again after 60 min (Table 1). Plasma [Cl^-] (143.6 mequiv.·L^{-1} immediately after capture) increased significantly after 30 min, but there was no further change after 60 min (Table 1). Plasma [K^+] (4.0 mequiv.·L^{-1} immediately after capture) decreased significantly after 30 min, but there was no further change after 60 min (Table 1).

Plasma metabolites also changed significantly during the recovery period. Plasma lactate (11.5 mmol·L^{-1} immediately after capture) increased significantly after 30 min and again after 60 min (Table 1). Plasma glucose (6.16 mmol·L^{-1} immediately after capture) increased significantly after 30 min, but there was no further change after 60 min (Table 1). Muscle metabolites (54.6 mmol lactate·kg^{-1}, 2.35 mmol glucose·kg^{-1}, 2.04 mmol glycogen·kg^{-1}, and 2.36 mmol PCr·kg^{-1}) did not change significantly after either the 30- or 60-min recovery periods (Table 1).

Net pen recovery

Of the 125 coho salmon placed in the net pen, only three were dead after 24 h (2.4% mortality). There were no significant differences among the three gear types for either plasma lactate or plasma glucose following a 24-h net pen recovery (Fig. 1). For all gear types, plasma lactate decreased significantly to 4–5 mmol·L^{-1} from 19–30 mmol·L^{-1} following a 24-h net pen recovery (Fig. 1). Plasma glucose values were unchanged after a 24-h net pen recovery (Fig. 1).

Physiological differences among gear types at time of capture

The data for the overall effect of gear type on the physiological variables with zero recovery time are summarized in Table 2. Among the hematological variables, plasma [Na^+], plasma [Cl^-], and plasma lactate were all significantly higher for gillnet gear compared with seine gear and troll gear (Table 2) but did not differ between either gillnet gear and troll gear or between seine gear and troll gear. Plasma osmolality was significantly higher for gillnet gear compared with seine gear and for gillnet gear compared with troll gear but not for troll gear compared with seine gear (Table 2). Plasma glucose, plasma [K^+], and Hct did not change significantly between gear types. With the exception of muscle lactate, none

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Table 4. Mature coho salmon plasma and muscle variables: a comparison with the visual fish condition ratings of 1 and 3.

	Rating[a]		
Response variable	1	3	Level of significance: 1 vs. 3[b]
Plasma Cl^- (mequiv.·L^{-1})	149.2	150.4	ns
Plasma Na^+ (mequiv.·L^{-1})	181.5	187.9	**
Plasma K^+ (mequiv.·L^{-1})	3.5	4.0	ns
Osmolality (mosmol)	371.9	389.9	***
Hct (%)	48.2	48.2	ns
Plasma lactate (mmol·L^{-1})	15.6	22.9	***
Plasma glucose (mmol·L^{-1})	7.4	7.8	ns
Muscle lactate (mmol·kg^{-1})	54.3	52.1	ns
Muscle glucose (mmol·kg^{-1})	2.45	2.28	ns
Muscle glycogen (mmol·kg^{-1})	1.73	1.45	ns
Muscle PCr (mmol·kg^{-1})	1.79	2.68	ns

[a]Least squares means. *N* values varied as follows: 224 and 133 for condition 1 plasma and tissue variables, respectively, and 62 and 38 for condition 3 plasma and tissue variables, respectively.
[b]Single-factor analysis of variance (model 1) ($\alpha = 0.05$ with additional protection for multiple comparisons): ns, not significant; $^{**}p < 0.01$; $^{***}p < 0.001$.

of the muscle metabolites (glucose, glycogen, and PCr) differed significantly between gear types (Table 2). Muscle lactate for gillnet gear was significantly lower than for seine gear but did not differ from troll gear (Table 2).

Physiological differences within gear types at time of capture

The data for the physiological effects within gear types are summarized in Table 3. Among the hematological variables and within gear types, no significant differences were detected among fishing methods for Hct, plasma [Na^+], plasma [K^+], plasma osmolality, plasma lactate, or plasma glucose. Plasma [Cl^-] was significantly higher for the ramped seine compared with the other seine methods. Among the muscle metabolites and within gear types, glucose, glycogen, and PCr did not differ significantly between fishing methods (Table 3). However, a 60-min gillnet soak time resulted in a significantly higher muscle lactate concentration compared with a 30-min gillnet soak time (Table 3). Differences between fishing methods for all gear types were few, as indicated in Table 3.

Were the effects of fishing method and holding time independent of each other?

Significant interactions were found between fishing method and recovery time for plasma [Cl^-] ($p < 0.0001$), plasma K^+ ($p < 0.002$), plasma lactate ($p < 0.023$), muscle glucose ($p < 0.039$), and muscle lactate ($p < 0.036$) concentrations. Therefore, the behaviour of these variables with recovery time depended on the specific fishing method, as illustrated in Fig. 2. For plasma [Cl^-], there were lower values at zero recovery time with seine brailing methods compared with the other fishing methods (Fig. 2A). For plasma lactate, there was a larger increase with recovery time for the troll gear (Fig. 2B). For fish caught by gill net with a 60-min soak time and by troll methods, muscle lactate levels increased rather than decreased with recovery time (Fig. 2C).

Physiological characteristics associated with the visual condition rating of fish

Only fish with visual condition ratings of 1 and 3 were statistically analyzed for differences in physiological status immediately after capture (Table 4). Plasma [Na^+], plasma osmolality, and plasma lactate were all significantly higher in fish visually ranked as 3 compared with those ranked as 1 (Table 4). None of the other variables were significantly different.

Discussion

The present study is the first to broadly examine the physiological status of wild coho salmon immediately after capture by three different types of commercial salmon fisheries. In addition, the physiological status of coho salmon held in recovery boxes onboard commercial fishing vessels or of coho salmon held in a net pen has not been followed previously. Our conclusions have a number of caveats. Foremost, they only apply to coho salmon that were not bleeding. Second, because coho salmon are normally commercial bycatch and we targeted coho salmon, there may be concerns about the application of specific aspects of our data to a normal commercial fishery. Third, potential vessel-to-vessel differences (including crews) were not thoroughly assessed in the analysis because of low *N* values for some seine gear and all troll gear. However, no statistically significant vessel-to-vessel differences were found for the gillnet samples (data not presented). Notwithstanding these concerns, the physiological measurements made in this study suggest that the coho salmon were severely exhausted immediately upon capture, regardless of fishing method.

Laboratory studies with hatchery-reared and wild fish have documented the range of acid–base, osmotic, electrolyte, and metabolite disturbances associated with exhaustive exercise (Milligan 1996). We used these literature values as a point of reference in the absence of control fish samples. As shown in the following section, the physiological disturbances in the present study were as extreme as have been measured previously in exhausted fish. Nevertheless, the low plasma lactate values suggest that physiological recovery after commercial capture was possible in a net pen, even though muscle metabolite measurements are needed to establish the extent of the recovery. Moreover, the net pen fish

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experienced a low incidence of delayed mortality after 24 h (2.4% this study) and after 48 h (3.35% in a companion study with the same stock; B. Hargreaves, Fisheries and Oceans Canada, Vancouver, B.C., unpublished data). Most postcapture mortality in salmonids is expected to occur within the first 6–24 h of holding (Wertheimer 1988).

Physiological status upon capture and the effect of the recovery boxes

Most fish were visually rated as nonbleeding and had an overall Hct of approximately 50%, and only three of the 303 coho salmon having a Hct below the normal range for salmonids (Gallaugher and Farrell 1998) confirmed this. By comparison, routine Hct for mature Alberni Inlet sockeye salmon was 28.8–33.8% for cannulated fish (Farrell et al. 1998), whereas exhausted fish sampled by acute venopuncture had a Hct of 43.2% (K. Tierney and A.P. Farrell, Simon Fraser University, Burnaby, B.C., unpublished data). It is important for the nonretention policy that commercial capture and handling of coho salmon can minimize bleeding because it might impair survival and migration after release, given that anemia reduces the critical swimming speed of salmonids (Gallaugher et al. 1995).

Muscle metabolites

Energy for skeletal muscle contraction is provided by the hydrolysis of PCr and ATP stores that decline precipitously with exhaustive, anaerobic exercise in fish (Milligan 1996). The extremely low levels of muscle PCr (<4.5 $mmol \cdot kg^{-1}$) in coho salmon immediately following capture with all gear types were comparable with those reported following exhaustive swimming in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) and after angling of Atlantic salmon.

However, we were surprised that the muscle PCr levels in coho salmon remained unchanged during the 1-h recovery period. Exhausted rainbow trout showed partial recovery of muscle PCr and ATP in <1 h (Pearson et al. 1990; Milligan 1996), as did wild Atlantic salmon during recovery from angling (Booth et al. 1995; Wilkie et al. 1996) and manual chasing (Wilkie et al. 1997).

To fuel glycolytic ATP production during anaerobic exercise, muscle glycogen is broken down to lactate. The muscle lactate values for coho salmon immediately following capture ranged between 43.6 and 62.5 $mmol \cdot kg^{-1}$. These muscle lactate values are as extreme as any observed previously either in Atlantic salmon after angling (Brobbel et al. 1996; Wilkie et al. 1996) or in rainbow trout after induced exercise (Milligan 1996). In rainbow trout, elevated muscle lactate levels can decline significantly after 1 h of recovery and routine levels are restored after 12 h (Kieffer et al. 1994; Milligan 1996). However, Stevens and Black (1966) showed for rainbow trout that prolonged recovery times were associated with very high muscle lactate levels (>56 $mmol \cdot kg^{-1}$). Similarly, it took 2 h of recovery before muscle lactate declined significantly after Atlantic salmon were angled (Booth et al. 1995; Wilkie et al. 1996). Brobbel et al. (1996) observed a similar slow decline in kelts but not in bright Atlantic salmon. Therefore, either an insufficient recovery time for the level of exhaustion or species differences could explain our finding that the muscle lactate levels did not change significantly after 1 h of recovery.

Muscle glycogen levels immediately following capture (1.13–4.24 $mmol \cdot kg^{-1}$) were comparable with those reported for rainbow trout following exhaustive exercise (e.g., Stevens and Black 1966; Pagnotta and Milligan 1991; Ferguson et al. 1993) and for Atlantic salmon following either angling (Booth et al. 1995; Brobbel et al. 1996; Wilkie et al. 1996) or manual chasing (Wilkie et al. 1997). While significant glycogen resynthesis can occur within 1–2 h following exhaustive exercise, we observed no glycogen resynthesis after 1 h of recovery. Temperature and the severity of the exhaustion apparently influence the rate of glycogen resynthesis. Slower glycogen resynthesis was reported for wild Atlantic salmon at 12°C compared with 18 and 23°C (Wilkie et al. 1997), and glycogen resynthesis was delayed when repetitive exhaustive exercise bouts caused a very large depletion of muscle glycogen (Stevens and Black 1966).

Muscle glucose levels were low (2.08–2.66 $mmol \cdot kg^{-1}$) and did not differ between gear types and methods, nor did they change significantly with recovery. Previous studies with exhausted rainbow trout showed either increases with recovery (Pearson et al. 1990; Pagnotta and Milligan 1991) or no change in muscle glucose levels (~4 $mmol \cdot kg^{-1}$; Wang et al. 1994).

Plasma variables

Plasma lactate levels continue to increase after exhaustion because a portion of the accumulated muscle lactate is released progressively into the blood stream (Milligan 1996). A similar process likely accounted for the increase in plasma lactate in coho salmon with recovery time for all fishing methods. In laboratory experiments, plasma lactate reaches a peak level (12–20 $mmol \cdot L^{-1}$) in about 2 h, after which plasma lactate declines more gradually to return to a routine level (<2 $mmol \cdot L^{-1}$) about 12 h into recovery (Wood et al. 1983; Milligan 1996). Plasma lactate values for coho salmon immediately after capture (11.5 $mmol \cdot L^{-1}$) and after a 60-min recovery period (23.2 $mmol \cdot L^{-1}$) were clearly at the upper end of the range for rainbow trout, again suggesting that the coho salmon were severely exhausted by commercial fishing. Similar plasma lactate values were reported for troll-caught coho salmon (Parker et al. 1959) and chinook salmon (Parker and Black 1959) but were lower for Atlantic salmon that were anaesthetized after angling (Booth et al. 1995; Brobbel et al. 1996; Wilkie et al. 1996) and for mature sockeye salmon following two consecutive critical swimming tests in a swim tunnel (Farrell et al. 1998; Jain et al. 1998). The low plasma lactate level in coho salmon after a 24-h net pen recovery is a novel finding. Given that the procedures used to sample fish from the net pen would have elevated plasma lactate levels, the plasma lactate values reported for net pen fish may underestimate the actual level of recovery.

Ion imbalances occurring with exhaustive swimming could contribute to muscle fatigue (see Wood et al. 1983; Holk and Lykkeboe 1998) and postexhaustion mortality (Parker et al. 1959; Wood et al. 1983). Previous studies showed that exhaustive swimming in seawater causes osmolality to increase during the first hour of recovery, with pre-exercise levels being restored within 24 h. A similar range of

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osmolality values has been observed in wild Atlantic salmon after manual chasing and during recovery (Wilkie et al. 1997). Also, coho salmon plasma [Na^+] (165–180 mequiv.·L^{-1}), [Cl^-] (141–147 mequiv.·L^{-1}), and [K^+] (3.7–4.3 mequiv.·L^{-1}) were similar to those observed in exhausted in wild Atlantic salmon (Booth et al. 1995) and in rainbow trout (Wood et al. 1983). Booth et al. (1995) observed that elevated plasma ion levels returned to preexercise levels after a 2-h recovery, and Wood et al. (1983) observed significant recovery for Na^+ and Cl^- but not K^+ levels after a 1-h recovery. In the present study, plasma Na^+ and Cl^- levels did not recover, but K^+ did recover after 30 min. Whether these ionic and osmotic disturbances reflect dehydration, increased ion uptake, or some combination is unclear from the present experiments, but the changes were likely associated with exhaustion because skin, scale, or gill damage was minimal.

We can offer four potential explanations why there was minimal metabolic, ionic, or osmotic recovery. First, the severity of exhaustion in wild fish may dictate a recovery period longer than 1 h. However, we did observe that the lethargic fish regained balance and began to roil around, sometimes struggling, in the recovery box. Second, this continued activity in the recovery box might have either reexhausted some fish or slowed metabolic recovery. This might explain the high individual variability among PCr values. Third, any struggling associated with dipnetting fish from the recovery box for tissue sampling might have lowered PCr (Wang et al. 1994; Milligan 1996). While we felt that muscle sampling in <45 s was as good as could be achieved with limited space and variable weather onboard commercial fishing vessels, a less stressful means to sample fish would be useful in eliminating this potentially confounding effect. Fourth, the current design for the recovery box may not optimize recovery. The recovery box provided room for coho salmon to roil and struggle during recovery, whereas for laboratory experiments, they are designed to limit fish movement and orient the fish into flowing water. Therefore, before reaching firm conclusions on the potential benefit of recovery boxes onboard commercial vessels, we suggest that experiments be performed with a redesigned recovery box that takes these factors into account, especially since slow swimming into a water current can promote a more rapid recovery in rainbow trout (Milligan 1996).

Delayed mortality and swimming performance after live release

Given the high level of metabolic exhaustion after commercial capture, there is a concern about delayed mortality among released fish. However, there was no coho salmon mortality in the recovery boxes and only 2.4% coho salmon mortality after 24 h in a net pen.

Delayed mortality following severe exertion was first shown for fish over 60 years ago, and postcapture mortality rates can be considerably higher (up to 70%) than we observed here. The proximate cause of delayed mortality is unknown. One prediction, however, is that lower levels of physiological disturbance during capture and handling promote better survival upon release. Indeed, troll-caught coho salmon that survived in live boxes had a lower plasma lactate level compared with those that showed delayed mortality (Parker et al. 1959).

Other concerns regarding the live salmon release include the possibility that they cannot escape predators, they cannot swim well enough to complete their migration, or they fail to reproduce successfully when they reach the spawning grounds. Again, such concerns need to be addressed before any final recommendations are made regarding the acceptability of any nonretention fishery methods. Issues such as the negative effects of stress on reproductive processes (Pankhurst and Dedual 1994) and the diversion of energy away from gonad development (Jonsson et al. 1991) are beyond the scope of our data. Furthermore, the ability of fish to swim immediately after commercial capture has not been rigorously studied and the coho salmon tag-and-release studies that were performed to assess migration success will be reported elsewhere. Therefore, we limit ourselves here to speculation on swimming ability as inferred from the fish's metabolic status.

Laboratory studies have related high muscle lactate levels with a compromised burst swimming ability in rainbow trout. Muscle lactate recovered to around 24 mmol·kg^{-1} after approximately 1 h following a 15-s burst of swimming, and fish could swim repeatedly provided muscle lactate was <45 mmol·kg^{-1} (Stevens and Black 1966). However, if muscle lactate was >56 mmol·kg^{-1}, muscle lactate did not recover over the next 1 h and the fish could (or would) not burst swim again. Muscle lactate in coho salmon was >43 mmol·kg^{-1}, reaching 63 mmol·kg^{-1} in some fish, and was therefore within the range that might impair burst swimming ability. Paulik et al. (1957) reported that adult coho salmon required several hours to recover swimming capacity after an initial exhaustive swimming bout but did not measure lactate levels. Similar to muscle lactate, plasma lactate levels >15 mmol·L^{-1} were suggested as an index of impaired critical swimming ability for mature, wild sockeye salmon (Jain et al. 1998). Therefore, the high plasma lactate levels in coho salmon (7.7–31.4 mmol·L^{-1}) also suggest impaired swimming ability. Even so, the concept of a metabolic threshold for repeat swimming may need refining because wild sockeye salmon can repeat critical swim speed tests without full metabolic recovery and only a 45-min recovery period (Farrell et al. 1998). Clearly, swimming performance studies on captured wild coho salmon are needed to properly assess their swimming potential when released after commercial capture.

Assessing the effects of commercial fishing gear types and methods on physiological stress

An inherent danger of such comparisons, especially when all fish were severely exhausted and differences among and within fishing gear types and methods were small on an absolute scale, is that more than one factor differed, making it difficult to assign specific cause–effect relationships. For example, the degree of air exposure varied considerably; troll-caught fish were stunned while on the hook at the water level, whereas fish caught by gill net and seine were air-exposed before being stunned. Ferguson and Tufts (1992) demonstrated that a 60-s air exposure following an exhaustive swim increased plasma lactate and a slowed recovery compared with those fish not experiencing air exposure. In-

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terestingly, coho salmon caught by troll gear had a significantly lower plasma lactate but not a lower muscle lactate compared with fish caught with other fishing gear. This significant difference could reflect the extent of air exposure.

The significantly higher levels of plasma lactate for gillnet-caught fish compared with seine gear caught fish could reflect a greater level of exhaustion during gillnet capture. However, this explanation is inconsistent with the lower muscle lactate concentrations at capture for gillnet gear versus seine gear. Instead, a more plausible explanation is that fish became exhausted well before they came onboard the gillnet vessel, and so the time-dependent lactate diffusion from the muscle into the blood stream had progressed further compared with seine and troll fishing.

Our observation that within seine gear types, the procedure of ramping was more stressful than for brailing methods is consistent with an earlier observation that mortality rates for all species of seine-caught salmon (including coho) were higher with ramping than with brailing (J.O. Thomas and Associates, Ltd. 1997). Also, a 60-min soak time for the gillnet fishery proved to be more stressful than a 30-min soak time based on muscle lactate differences.

In conclusion, extensive physiological measurements suggest that coho salmon experienced severe metabolic disruption regardless of gear types and methods, and differences in the physiological condition of coho salmon among and within fishing gear types were relatively small. With the exception of plasma K^+, most of the variables that we measured did not recover when fish spent up to 1 h in a recovery box onboard the commercial fish vessel. Therefore, we have reservations about the adequacy of the recovery box design in terms of promoting optimal recovery and feel that it should be redesigned to seek improvements. In contrast, a 24-h recovery in a net pen promoted recovery of plasma lactate and resulted in a low level of delayed mortality.

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Spatial and dietary overlap in the Georges Bank groundfish community

Lance P. Garrison

Abstract: Patterns in spatial and trophic resource partitioning in the fish community of the Georges Bank region are identified, accounting for size-based changes in diets. During autumn and spring, this community is divided into geographic assemblages of species that have high spatial overlap. Similarity in spatial distribution is primarily related to similarity in depth preferences, and seasonal differences in species composition within assemblages are related to migrations. There is also important trophic structure within the Georges Bank community separating predators based upon prey size and location in the water column. Ontogenetic changes in diets are an important feature of the trophic structure in this system, particularly in the major piscivores. Seasonal changes in trophic structure reflect both predator and prey migrations. Dietary overlap among predator types is independent of either spatial overlap or depth preferences. There is spatial segregation within trophic guilds, and this spatial partitioning reduces the potential for exploitative competition within this community. Given the observed spatial patterns in trophic structure, the geographic assemblages on Georges Bank may be considered ecologically distinct functional units within this ecosystem.

Résumé : J'ai identifié sur le banc Georges les modes de répartition spatiale et trophique de la communauté de poissons en tenant compte des changements dans le régime alimentaire en fonction de la taille. Pendant l'automne et le printemps, cette communauté est divisée en assemblages géographiques d'espèces qui présentent un fort chevauchement spatial. La similarité dans la distribution spatiale est liée avant tout à la similarité dans les préférences de profondeur, et les différences saisonnières dans la composition spécifique des assemblages sont liées aux migrations. On note aussi sur le banc Georges une forte structure trophique qui sépare les prédateurs en fonction de la taille de leurs proies et de leur place dans la colonne d'eau. Les changements ontogénétiques dans le régime alimentaire sont une caractéristique importante de la structure trophique de ce système, particulièrement chez les grands piscivores. Les changements saisonniers dans la structure trophique reflètent les migrations à la fois des prédateurs et des proies. Le chevauchement trophique entre les types de prédateurs est indépendant aussi bien du chevauchement spatial que des préférences de profondeur. On observe une ségrégation spatiale à l'intérieur des guildes trophiques, et cette répartition spatiale réduit le potentiel d'exploitation concurrentielle au sein de cette communauté. Étant donné les modes spatiaux observés de la structure trophique, les assemblages géographiques du banc Georges peuvent être considérés comme des unités fonctionnelles écologiquement distinctes au sein de ce système.

[Traduit par la Rédaction]

Introduction

The global crisis in overexploitation of fisheries has resulted in calls for multispecies and (or) "ecosystem-based" assessment and management of fish stocks and assemblages (Jennings and Kaiser 1998; National Research Council 1999). This stems from the perception that more effective management requires improved understanding of the ecological mechanisms controlling population dynamics of exploited stocks (Larkin 1996; National Research Council 1999). These include species interactions (e.g., predation and competition), changes in resource availability, or environmental variation (Fogarty and Murawski 1998; Jennings and Kaiser 1998). Implementation of multispecies approaches requires improved understanding of the community ecology of fish assemblages that has rarely been explicitly incorporated into management practices.

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L.P. Garrison. Northeast Fisheries Science Center, National Marine Fisheries Service, 166 Water St., Woods Hole, MA 02543, U.S.A. e-mail: Lance.Garrison@noaa.gov

Within the last three decades, the Georges Bank fish assemblage has experienced major changes in species composition associated with fishery exploitation (Fogarty and Murawski 1998). The most notable changes are significant declines in commercially exploited gadids and flatfish, an increase in elasmobranchs (particularly spiny dogfish), and recent dramatic increases in the biomass of small pelagic fish (e.g., Atlantic herring, Fogarty and Murawski 1998). While the decline in heavily exploited species is generally attributed to fishery practices, the concomitant increase in less exploited groups is often associated with putative indirect effects of fishing and species interactions (Fogarty and Murawski 1998). The hypotheses concerning species interactions have generally received little direct examination and remain largely untested.

Spatial and temporal patterns in species distributions mediate potential trophic interactions. In continental shelf fish communities, environmental gradients (e.g., temperature and depth) influence patterns of species codistribution (Murawski and Finn 1988) and result in regional and subregional geographic assemblages (Overholtz and Tyler 1985; Gabriel 1992; Gomes et al. 1995). Seasonal and interannual changes in species distributions and abundances alter the

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species composition within these assemblages; however, the geographic boundaries are consistent over decadal time scales (Gabriel 1992; Gomes et al. 1995). Due to their association in space, species in the same assemblages are likely to interact strongly with one another. Spatial assemblages of species are therefore potentially important ecological units, may have distinct trophic structure, and may respond differently to human activities, environmental variation, or changes in food resources.

Fish body sizes frequently span four orders of magnitude across life history, often resulting in discrete ontogenetic shifts in both habitat use and diet (Werner and Gilliam 1984; Ross 1986). The trophic relationship between species may therefore change through ontogeny, and the degree of niche overlap between two species may also vary ontogenetically (Polis 1984; Piet et al. 1999). An explicit incorporation of these size-based differences in resource use is essential to evaluating the spatial and trophic structure of fish assemblages (e.g., Piet et al. 1999).

In this study, I examined the spatial and trophic structure within the fish assemblage of the Georges Bank region and the relationship between habitat, spatial, and dietary overlap accounting for size-based changes in resource use. Resource partitioning along both spatial and trophic gradients was evaluated as well as seasonal variation associated with changes in species distributions and resource availability. Given the observed patterns in spatial and trophic structure, I explore the potential for strong competitive interactions in this community and discuss the implications for ecosystem-based management and assessment on Georges Bank.

Methods

Data sources

I utilized data from bottom trawl surveys conducted by the Northeast Fisheries Science Center (NEFSC). The survey employed a stratified random sampling design with strata defined by depth and latitude. Sample depths ranged from 8 to 400 m. For each tow, catch at length (1 cm length-classes) was recorded for all captured species. Details of the survey sampling design, execution, and efficiency are available in Azarovitz (1981) and NEFSC (1988).

In addition to catch data, a subset of species were analysed for food habits data during each cruise. In general, one individual fish from each 1-cm size-class of a given target species was examined at each station. Fish were measured and weighed, total stomach volume was measured, and the proportional volume of each prey type in the stomach was quantified. Prey items were identified at sea to the lowest possible taxonomic level, focusing on the identification of fish prey to species. Details of the food habits sampling methodology are available in NEFSC (http://www.nefsc.nmfs.gov/pbio/fwpd). In the current analysis, fish and squid prey were retained at the species level, while invertebrate prey items were typically lumped into family or order groups as appropriate.

The current study included the Georges Bank region as defined by boundaries in faunal assemblages (Gabriel 1992; L.P. Garrison, unpublished data) and included survey strata corresponding to the shelf region south of Cape Cod, Georges Bank, and Stellwagen Bank (Fig. 1). The time frame included spring (March–April) and autumn (September–October) from 1991 to 1997. These seasons were best represented in the NEFSC surveys. During this period, 865 trawls were collected during the autumn, while 781 trawls were collected during spring.

Predator categories

Twenty-seven predator species were evaluated, including two squid species (Table 1). The predator species were divided into length categories to account for ontogenetic shifts in diet based upon historical data and previous analyses (Bowman and Michaels 1984; Garrison and Link 2000*a*). Only those species/size-classes with greater than 20 nonempty stomachs sampled were included in the diet analyses.

Resource overlap index

Overlap in resource use along spatial, trophic, and environmental gradients in the Georges Bank fish community was examined. Schoener's (1970) index of resource similarity was used for all three gradients:

$$(1) \qquad O_{ij} = 1 - 0.5(\Sigma|p_{ik} - p_{jk}|)$$

where p is the proportion of resource state k for predator types i and j. In all cases, resource overlap was assessed between predator types as defined by the species/size-class combinations noted above (Table 1).

Abundance data from the NEFSC bottom trawl surveys were used to identify spatial assemblages in the Georges Bank fish community. The mean biomass of each predator type (species/size-class combination) within 0.25° square grid cells (area = 588 km^2) was calculated for each season. The spatial cells were then clustered based upon their similarity in species composition using hierarchical agglomerative clustering methods (Pielou 1984). In addition, spatial gradients in species composition were examined using a detrended correspondence analysis (DCA, Hill and Gauch 1980) also based upon 0.25° cells. A spatial overlap matrix between predators was also calculated using the Schoener index (see eq. 1 above where each grid cell is a resource state k and p_{ik} is the proportion of the biomass of predator i in cell k).

Hierarchical agglomerative clustering methods were also used to identify groups of species with similar diets (Pielou 1984). Resource overlap was quantified using the Schoener index (eq. 1) where p_{ik} was the mean proportional volume of a prey category k in the diet of predator type i.

Finally, I calculated overlap in habitat similarity based upon the depth of occurrence for each species/size-class. Depth is the primary environmental factor driving spatial structure in fish communities in northern habitats and Georges Bank (Overholtz and Tyler 1985; Murawski and Finn 1988). Catch data was restratified into 10-m depth intervals, and the mean biomass of each species/size-class within each depth interval was calculated. Overlap in habitat similarity was calculated as above (eq. 1) where p_{ik} is the mean proportional biomass of predator type i within depth interval k.

Partial Mantel regression and path analysis

To investigate the relationship between depth overlap (Z_{ij}), spatial overlap (S_{ij}), and diet overlap (D_{ij}), I employed partial Mantel regressions after the method described in Smouse et al. (1986) and path analysis described in Legendre and Legendre (1983) and Legendre and Troussellier (1988). The Mantel correlation index between matrices (Mantel 1967) assesses the proportion of variance in one overlap matrix that is explained by another in a manner analogous to both Pearson correlation and linear regression methods (Smouse et al. 1986). The partial Mantel regression evaluates the relationship between two matrices after accounting for the mutual correlation with a third. In the current application, this was accomplished by first calculating the Mantel correlations between the diet (**D**), spatial (**S**), and depth (**Z**) overlap matrices. Then, the residual matrices were calculated, e.g., **Z**|**S** and **D**|**S**, which are the residual values after the regression of **Z** on **S** and **D** on **S**, respectively. Finally, the Mantel correlation ($R_{zd|s}$) of **Z**|**S** on **D**|**S** was

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Fig. 1. Map of Georges Bank region showing the bathymetry.

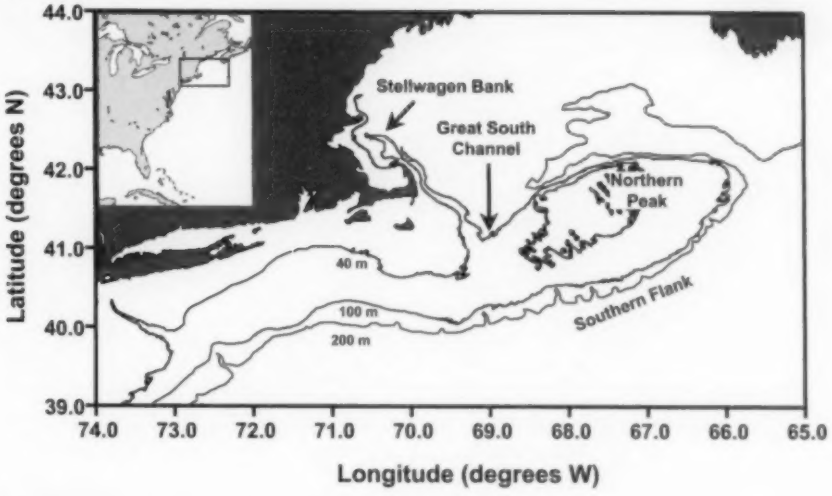

Table 1. Length categories for fish and squid species included in the current study.

		Length category (cm)			
Scientific name	Common name	Small	Medium	Large	Extra large
Squalus acanthias	Spiny dogfish	10–36	37–80	>80	—
Raja ocellata	Winter skate	10–30	31–60	61–80	>80
Raja erinacea	Little skate	10–30	31–60	61–80	>80
Raja radiata	Thorny skate	10–30	31–60	61–80	>80
Clupea harengus	Atlantic herring	10–20	21–30	>30	—
Merluccius bilinearis	Silver hake	10–20	21–40	>40	—
Gadus morhua	Atlantic cod	10–20	21–50	51–80	>80
Melanogrammus aeglefinus	Haddock	10–20	21–50	51–80	>80
Pollachius virens	Pollock	10–20	21–50	51–80	>80
Urophycis tenuis	White hake	10–20	21–40	>40	—
Urophycis chuss	Red hake	10–20	21–40	>40	—
Urophycis regia	Spotted hake	10–20	21–40	>40	—
Paralichthys dentatus	Summer flounder	10–20	21–40	41–70	>70
Paralichthys oblongus	Fourspot flounder	10–20	21–40	41–70	>70
Pleuronectes ferrugineus	Yellowtail flounder	10–20	21–40	41–70	>70
Pleuronectes americanus	Winter flounder	10–20	21–40	41–70	>70
Scophthalmus aquosus	Windowpane	10–20	21–40	41–70	>70
Scomber scombrus	Atlantic mackerel	10–20	21–35	>35	—
Peprilus triacanthus	Butterfish	10–20	21–35	>35	—
Pomatomus saltatrix	Bluefish	10–30	31–70	>70	—
Myoxocephalus octodecemspinosus	Longhorn sculpin	10–25	26–50	>50	—
Hemitripterus americanus	Sea raven	10–25	26–50	>50	—
Ammodytes dubius	Northern sand lance	—	10–25	>25	—
Macrozoarces americanus	Ocean pout	10–30	31–60	61–90	>90
Lophius americanus	Goosefish	10–30	31–60	61–90	>90
Illex illecebrosus	Shortfin squid	10–15	16–30	>30	—
Loligo pealleii	Longfin squid	10–15	16–30	>30	—

computed to evaluate the relationship between the depth and diet matrices after accounting for the influence of the spatial matrix (Smouse et al. 1986).

The partial Mantel regression was used to conduct a path analysis of the relationships between the spatial, depth, and dietary overlap matrices (Legendre and Legendre 1983; Legendre and Troussellier 1988). Path analysis identifies the causal pathways between multiple explanatory factors. The statistical significance of the Mantel coefficients was tested using permutation tests with 5000 iterations (Mantel 1967). Due to multiple testing, all significance tests were assessed at a Bonferroni-corrected alpha value (initial $\alpha = 0.05$, Legendre and Troussellier 1988).

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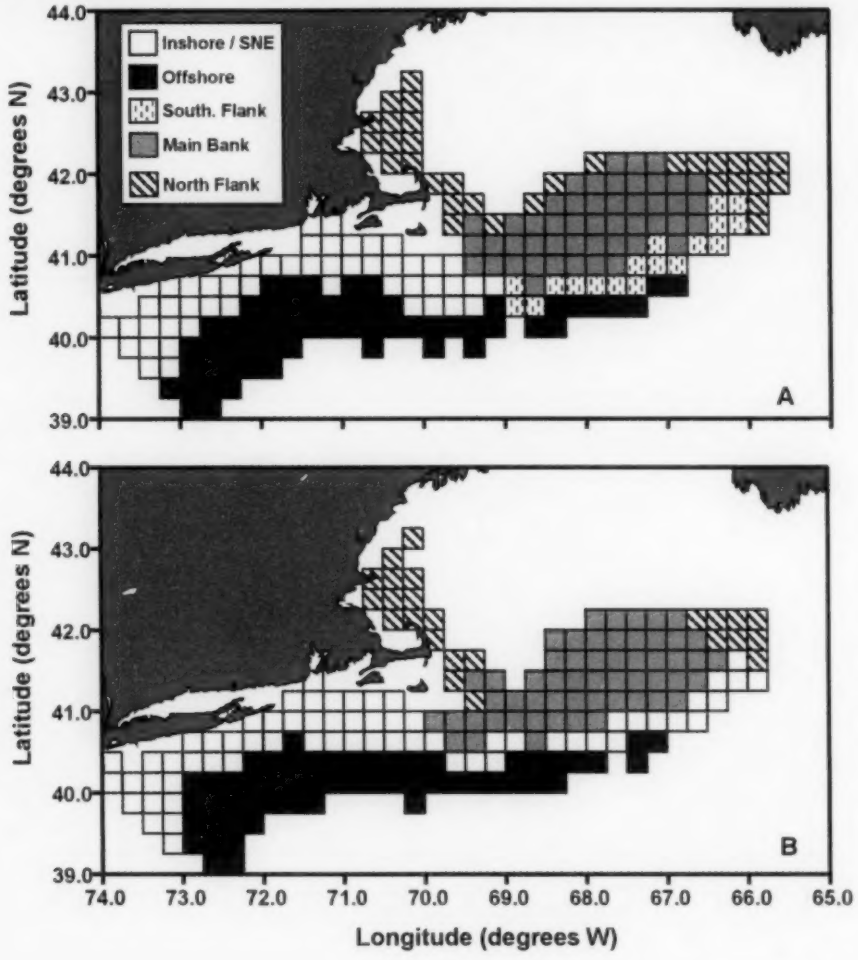
Fig. 2. Map of spatial assemblage structure in the Georges Bank region during (A) autumn and (B) spring.



Results

Spatial assemblages on Georges Bank

During autumn, there were five spatial assemblages on Georges Bank indicated by both the cluster analysis and DCA (Figs. 2A and 3A). The assemblages included inshore regions of southern New England (SNE), offshore habitats of southern New England and south of Georges Bank (OFF), deep habitats on southern Georges Bank (SGB), the main shallow portion of central Georges Bank (MGB), and the northeast tip and northern flank of the Georges Bank along with the Stellwagen Bank region (NFS) (Fig. 2A). These assemblage areas clustered together in the DCA space described by the first two ordination axes that explained 23.9% of the variance in the species abundance data (Fig. 3A). The first axis corresponded to a latitudinal gradient from southern to northern areas, while the second axis described a depth gradient from deep to shallow habitats (Fig. 3A).

The SNE assemblage in the autumn was dominated by medium and large spiny dogfish that together accounted for 80% of the captured biomass (Table 2). Spiny dogfish were not abundant in the OFF assemblage that was dominated by pelagic species including longfin squid (23.9%), small butterfish (26.8%), and Atlantic mackerel (9.4%) along with several hakes (Table 2). The SGB assemblage was characterized by a mix of pelagic and demersal species including medium spiny dogfish (22.6%), small butterfish (16.3%), medium little skate (6.8%), medium red hake (7.4%), and medium fourspot flounder (6.0%) along with demersal species such as longhorn sculpin, winter skate, and yellowtail flounder that were rare in the SNE and OFF assemblages (Table 2). In addition to medium spiny dogfish (12.6%), the MGB assemblage was dominated by primarily demersal species including medium, large, and extra large winter skate (totaling 28.2 %) and medium little skate (7.6%). The MGB assemblage was also characterized by northern species such as Atlantic cod, winter skate, windowpane, winter flounder, and sea raven (Table 2). Finally, the NFS assemblage was also dominated by spiny dogfish (medium and large, total 51.1%) but was characterized by high biomass of medium

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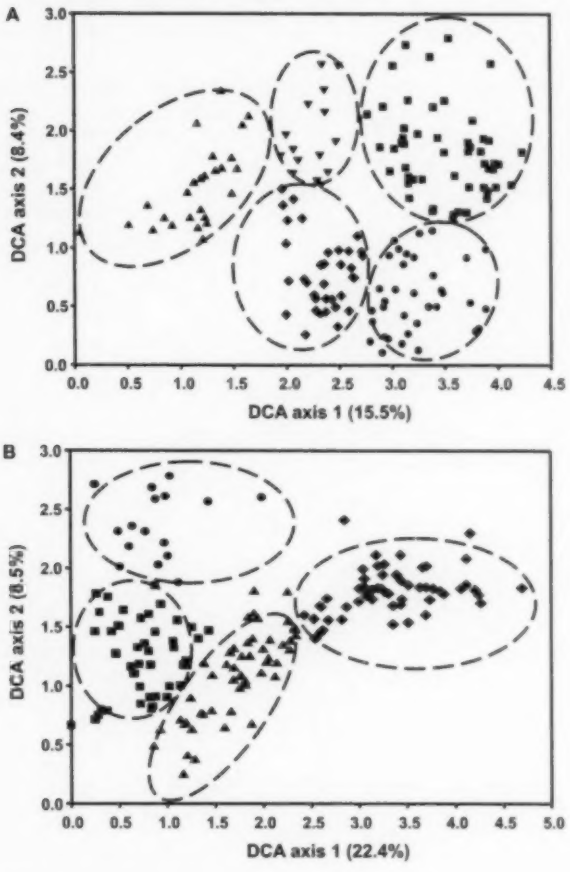
Fig. 3. DCA identifying spatial patterns in species composition on Georges Bank during (A) autumn and (B) spring. Each point in the DCA plot represents a 0.25° square grid cell. Symbols correspond to assemblages identified in Fig. 2: ▲, SNE; ◆, OFF; ▼, SGB (autumn); ■, MGB; ●, NFS.



Atlantic herring (9.6%) along with medium and large haddock (9.1%), in contrast with other assemblages (Table 2).

During spring, there were four spatial assemblages on Georges Bank that were identified in both the cluster analysis and DCA (Figs. 2B and 3B). The autumn SGB assemblage combined with the SNE assemblage during spring reflecting seasonal movements of migratory species such as spiny dogfish and Atlantic herring. The first two DCA axes accounted for 30.9% of the species variance. The first axis reflected a depth gradient from shallow to deep habitats, while the second axis reflected a latitudinal gradient from southern to northern regions (Fig. 3B).

The spring SNE and OFF assemblages were both dominated by medium and large spiny dogfish, accounting for 62.8 and 71.3% of captured biomass, respectively (Table 3). Small and medium Atlantic herring were also important in both assemblages, accounting for a total of 8.1% of the biomass in SNE and 4.8% in OFF. However, the SNE assemblage was also characterized by a high proportion of medium little skate (10.5%), while the OFF assemblage was characterized by medium silver hake (5.2%), Atlantic mackerel (medium and large, total 6.6%), small butterfish (2.3%), and longfin squid (total 2.5%). The MGB assemblage had a relatively low proportion of spiny dogfish (21.3%) and was characterized by northern demersal species including medium, large, and extra large winter skate (total 26%), large and medium haddock (total 13.9%), and large cod (7.6%) (Table 3). Finally, the spring NFS assemblage was dominated by medium and large spiny dogfish (total 34.9%) and the gadids including Atlantic cod (total of all size-classes 13.8%), haddock (total 7.8%), and pollock (total 13%) (Table 3).

Table 2. Species composition (percentage of biomass) in autumn spatial assemblages.

Species	SNE	OFF	SGB	MGB	NFS
M spiny dogfish	54.4	T	22.6	12.6	40.3
L spiny dogfish	23.8	T	—	4.4	10.8
M little skate	5.3	5.7	6.8	7.6	—
S longfin squid	3.4	23.9	1.2	4.9	—
S butterfish	2.7	26.8	16.3	4.6	T
M winter flounder	2.0	—	T	3.0	1.8
M fourspot flounder	1.1	3.3	6.0	T	—
S Atlantic mackerel	—	9.4	0.9	T	—
M shortfin squid	—	4.9	2.5	0.8	T
M silver hake	0.5	4.3	5.7	2.3	1.7
M red hake	T	3.9	7.4	1.5	2.7
M longfin squid	0.9	2.7	T	1.4	—
M goosefish	T	2.4	T	T	T
M spotted hake	T	2.2	T	—	—
S shortfin squid	—	2.1	T	T	T
M bluefish	0.7	1.4	—	1.1	—
S longhorn sculpin	—	T	5.0	1.5	1.5
M winter skate	0.7	T	4.2	9.1	1.1
M longhorn sculpin	—	—	4.0	2.8	3.8
M yellowtail flounder	T	T	3.0	2.2	0.7
XL winter skate	T	—	2.6	11.0	T
S spiny dogfish	—	T	2.4	—	—
L winter skate	T	—	2.0	8.1	0.8
M Atlantic herring	—	T	T	4.5	9.6
L Atlantic cod	—	—	T	2.2	3.2
M windowpane	T	—	—	2.1	—
M Atlantic mackerel	—	0.6	T	2.0	T
S Atlantic herring	T	—	—	1.5	T
M sea raven	T	—	0.7	1.1	1.0
L winter flounder	—	—	T	1.1	T
M haddock	—	—	T	T	6.7
L haddock	—	—	T	—	2.3
M Atlantic cod	—	—	—	T	2.0
M pollock	—	—	—	—	1.0

Note: Size-classes: S, small; M, medium; L, large; XL, extra large (see Table 1). Only species accounting for ≥0.5 % of biomass in any assemblage are included. T, <0.5% biomass; missing value, <0.01% biomass.

Dietary guilds on Georges Bank

Autumn diet groups

The autumn diet analysis identified nine species groups that clustered together at similarity levels greater than 40% (Fig. 4). These groups were broadly categorized into five

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Table 3. Species composition (percentage of biomass) in spring spatial assemblages.

Species	SNE	OFF	MGB	NFS
M spiny dogfish	47.8	48.5	14.7	22.1
L spiny dogfish	15.0	22.8	6.6	12.8
M little skate	10.5	0.7	8.2	1.0
M Atlantic herring	4.8	4.2	1.4	4.5
S Atlantic herring	3.3	0.6	T	T
M ocean pout	2.5	T	0.8	2.6
M winter skate	1.8	—	9.5	1.1
M silver hake	1.4	5.2	T	0.6
M longhorn sculpin	1.4	—	4.8	2.5
S little skate	1.2	—	T	—
L ocean pout	1.0	—	2.4	2.0
M Atlantic mackerel	0.6	3.8	T	T
L Atlantic mackerel	T	2.8	T	T
S butterfish	T	2.3	—	—
S spiny dogfish	—	1.9	—	—
M fourspot flounder	T	1.7	—	T
S longfin squid	—	1.5	—	—
M longfin squid	T	1.0	—	—
XL winter skate	T	—	8.9	1.4
L haddock	T	—	8.8	3.6
L winter skate	0.6	—	7.6	0.7
L Atlantic cod	0.6	—	7.0	5.5
M haddock	T	—	5.1	4.2
XL Atlantic cod	T	—	2.6	3.7
S longhorn sculpin	0.7	—	2.0	2.6
M yellowtail flounder	0.9	—	1.7	1.8
M sea raven	0.7	—	1.4	3.0
L pollock	—	—	T	7.5
M pollock	—	—	T	5.5
M Atlantic cod	—	—	0.8	4.6
M winter flounder	T	—	0.7	1.5

Note: Size-classes: S, small; M, medium; L, large; XL, extra large (see Table 1). Only species accounting for ≥0.5 % of biomass in any assemblage are included. T, <0.5% biomass; missing value, <0.01% biomass.

major trophic groups including piscivores, shrimp predators, demersal predators, planktivores, and crab predators (Fig. 4).

The piscivores included three groups (groups Ia–Ic). The major piscivores included large size-classes of spiny dogfish, Atlantic cod, several hake species, and all size-classes of goosefish and sea raven (group Ia) (Fig. 4). Their diets consisted primarily of unidentified fish (27%), Atlantic herring (11%), and silver hake (8%) (Fig. 5A). Large and medium pollock and large bluefish formed a distinct cluster (group Ib, Fig. 4), and their diets had high proportions of Atlantic herring (31%) in addition to northern sand lance (7%), squids (totaling 10%), and euphausiids (5%) (Fig. 5A). Large and extra large winter skate were also piscivorous (group Ic, Fig. 4), consuming northern sand lance (10%), silver hake (6%), and Atlantic herring (4%) in addition to benthic invertebrates including polychaetes (17%) and bivalves (16%) (Fig. 5A).

The shrimp predators included medium and small size-classes of demersal fish including hakes, little skate, longhorn sculpin, and Atlantic cod (group II, Fig. 4). These predators fed primarily upon shrimp taxa including pandalids (14%) and crangonids (8%) as well as other small pelagic prey such as silver hake (13%) and decapod larvae (5%). Their diets also included several benthic taxa such as *Cancer* crabs (7%) and gammarid amphipods (5%) (Fig. 5B).

The demersal predators included skates, flatfish, and haddock (group III, Fig. 4) that feed primarily upon benthic invertebrates. Their diets were composed of gammarid amphipods (16%) and polychaetes (10%) along with several other benthic taxa including isopods, asteroides, and *Cancer* crabs. These predators also consumed shrimp taxa, in particular crangonids (10%) (Fig. 5C).

The pelagic predators included the primary planktivores, Atlantic herring and Atlantic mackerel, along with small spiny dogfish (groups IVa and IVb, respectively, Fig. 4). The planktivores consumed a suite of small pelagic prey including larvaceans (17%), hyperiid amphipods (11%), euphausiids (10%), and mysids (9%) (Fig. 5D). Small spiny dogfish was unique in that a high proportion of its diet comprised ctenophores (42%) and cephalopods (16%) (Fig. 5D).

Finally, the crab predators included large demersal predators in two small groups separating medium summer flounder and extra large Atlantic cod (group Va) from large thorny skate (group Vb) (Fig. 4). Both Atlantic cod and summer flounder diets included a large proportion of *Cancer* crabs (55%) along with *Loligo* squid (16%) and other taxa (Fig. 5E). Large thorny skate diets included a very high proportion of polychaetes (69%) along with decapod crabs (16%) (Fig. 5E).

Spring diet groups

During spring, 10 trophic groups were identified that clustered together at a similarity level greater than 32% and were broadly categorized as shrimp/amphipod predators (group I), pelagic predators (groups IIa and IIb), demersal predators (groups IIIa and IIIb), generalists (groups IVa–IVc), and piscivores (groups Va and Vb) (Fig. 6).

The shrimp/amphipod predator group was a diverse suite of predators including small hakes, small Atlantic cod, several skate species, and demersal taxa such as longhorn sculpin and fourspot flounder (group I, Fig. 6). The diets of these species were very broad and included both benthic and pelagic invertebrates. Their diets primarily comprised gammarid amphipods (23%), pandalid shrimp (10%), crangonid shrimp (8%), polychaetes (5%), and *Cancer* crabs (5%) (Fig. 7A).

The second major group included both shrimp predators (group IIa) and planktivores (group IIb) that fed upon small pelagic prey (Fig. 6). The shrimp predators included windowpane and medium and large pollock, and their diets consisted of primarily euphausiids (33%), mysids (15%), and pandalid shrimp (10%) (Fig. 7B). The planktivores included both Atlantic herring and Atlantic mackerel whose prey were primarily copepods (21%) and larvaceans (11%) and included a high proportion of unidentified animal remains (34%) (Fig. 7B).

The demersal predators included flatfish, thorny skate, and haddock that fed primarily upon small benthic invertebrates (groups IIIa and IIIb, Fig. 6). The flatfish and skate (group IIIa, Fig. 6) consumed polychaetes (59%) and gammarid amphipods (12%), and haddock (group IIIb, Fig. 6) diets consisted primarily of amphipods (69%) and polychaetes (20%).

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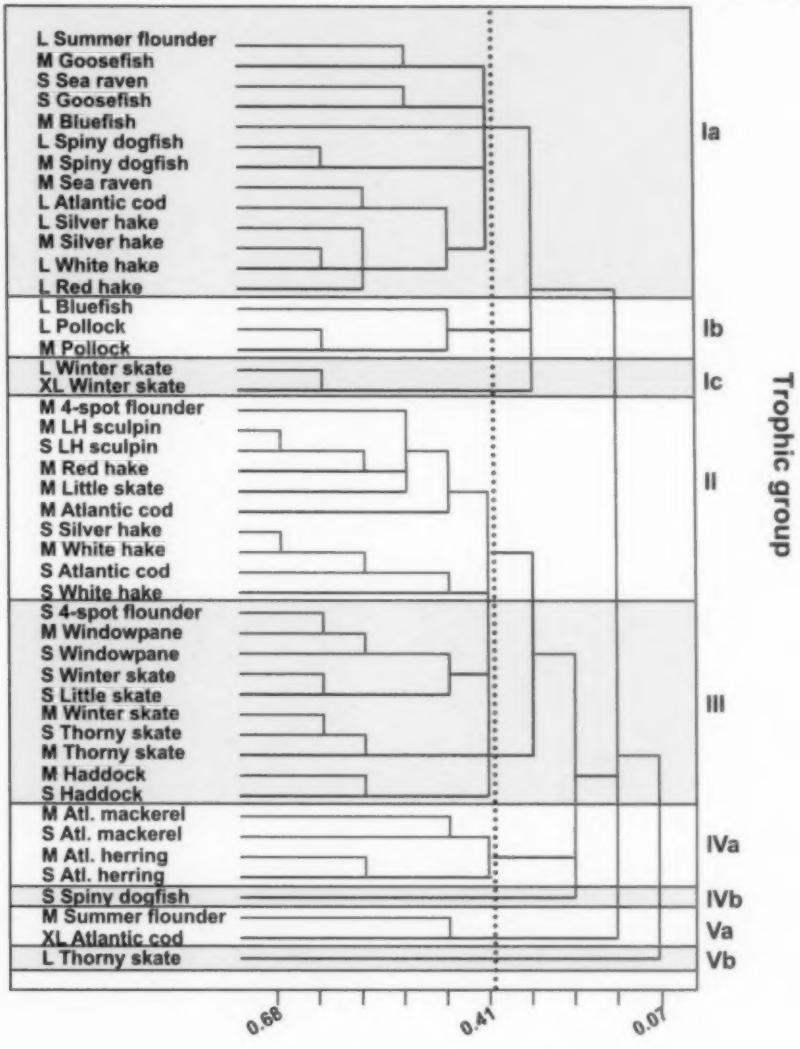
Fig. 4. Dendrogram of diet similarity (Schoener's index) during autumn. Trophic groups are identified at similarity levels >0.40.



The fourth major group included three clusters of generalist predators (groups IVa–IVc, Fig. 6). All three size-classes of spiny dogfish clustered together (group IVa, Fig. 6), reflecting the high proportion of ctenophores in their diets (38%) in addition to unidentified fish (15%), Atlantic herring (5%), clupeids (4%), and bivalves (4%) (Fig. 7D). Large and extra large winter skate also formed a distinct group (group IVb, Fig. 6) and preyed upon benthic taxa such as bivalves (31%) and polychaetes (17%) in addition to pelagic fish such as northern sand lance (4%) and clupeids (4%) (Fig. 7D). Medium, large, and extra large Atlantic cod (group IVc, Fig. 6) had very broad diets that were not dominated by any single prey item (Fig. 7D). Their diets included Atlantic herring (9%), clupeids (6%), scallops (7%), bivalves (6%), *Cancer* crabs (6%), decapod crabs (6%), and decapod larvae (4%) along with a variety of other benthic and pelagic prey items (Fig. 7D).

Finally, two groups of piscivores were identified during the spring (groups Va and Vb, Fig. 6). Medium and small sea raven (group Va) were distinguished from the other piscivores by the high proportion of benthic fish in their diets including ocean pout (27%), cusk-eel (3%), and flatfish (2%) (Fig. 7E). The major piscivores included medium and large hakes and all sizes of goosefish (group Vb), and their diets consisted primarily of silver hake (32%), unidentified fish (25%), and pandalid shrimp (11%) along with other fish (Fig. 7E).

Mantel regression and path analysis

The results of the Mantel regression analysis were similar

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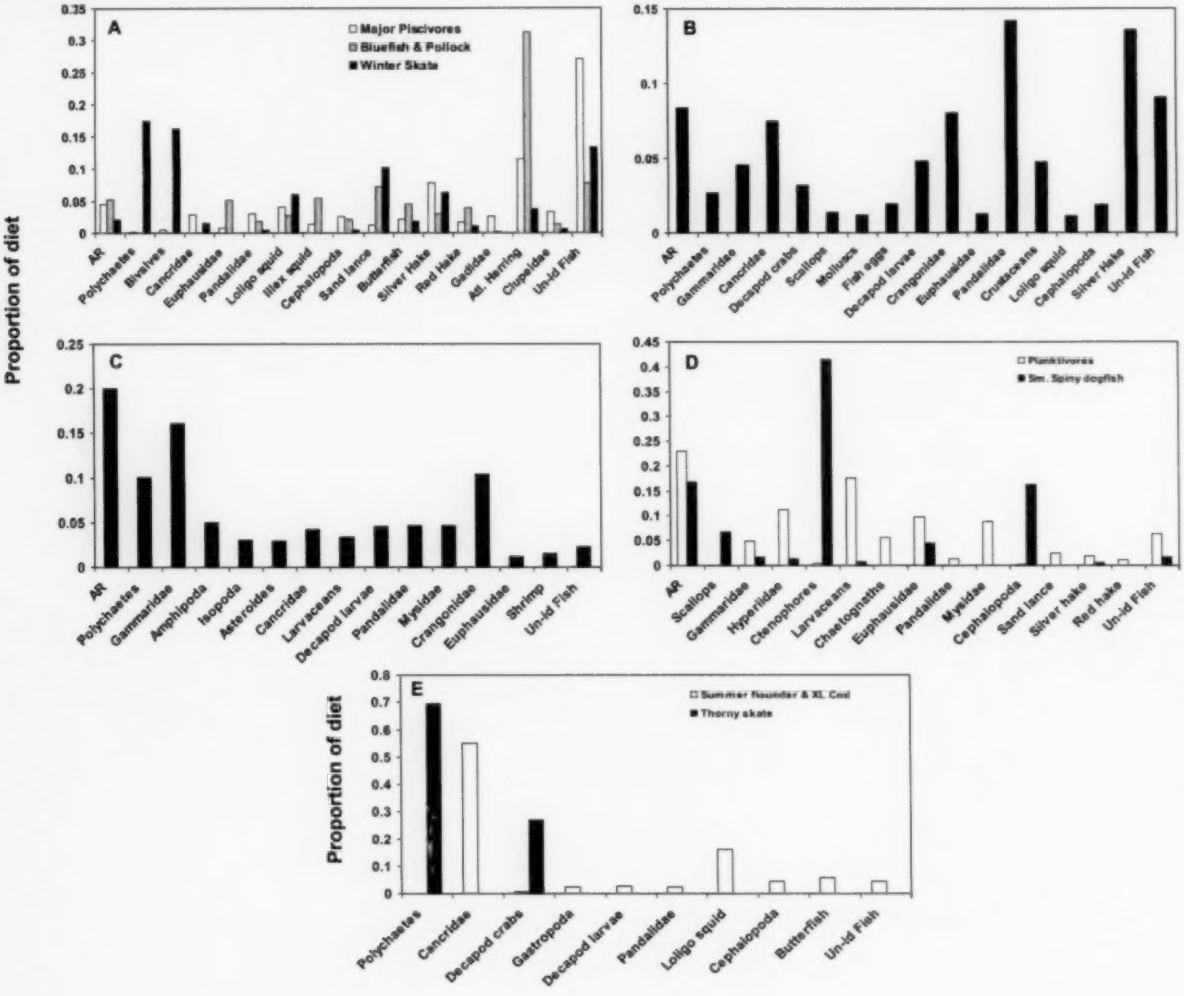
Fig. 5. Mean diet composition of five major trophic groups (Fig. 4) during autumn: (A) piscivores, (B) shrimp predators, (C) demersal predators, (D) pelagic predators, and (E) crab predators.



for both seasons. In both autumn and spring, approximately 58% of the variance in the spatial overlap matrix was explained by similarity in depth preferences among predators, and this was a highly significant, positive relationship (Table 4). In both seasons, the diet overlap matrix was unrelated to both the depth and spatial overlap matrices (Table 4). There was no correlation between similarity in the use of spatial resources and that of trophic resources.

Spatial and trophic resource partitioning

Patterns in spatial and trophic resource use were further examined by assessing the relative abundance of major trophic groups within spatial assemblages during each season (Fig. 8). There were strong spatial differences in the predominant trophic group within the Georges Bank region. During autumn, the SNE assemblage was dominated primarily piscivorous species (Fig. 8A), primarily large and medium spiny dogfish (Table 2). In contrast, the autumn OFF assemblage was dominated by small pelagic predators (Fig. 8A), primarily longfin squid, butterfish, and Atlantic mackerel (Table 2). Note that while small sample sizes in the current analysis precluded the inclusion of both squids and butterfish in the diet analyses, these species are pelagic predators feeding primarily upon small fish, squids, and zooplankton based upon data from earlier years where they were adequately sampled (Garrison and Link 2000*a*). Both the MGB and SGB assemblages contained a more diverse suite of predators including shrimp predators, piscivores, and pelagic predators (Fig. 8A). The SGB assemblage biomass was evenly distributed among piscivores (spiny dogfish), shrimp predators (medium silver and red hake and longhorn sculpin and medium little skate), and pelagic predators (butterfish) (Table 3). The MGB assemblage had a higher proportion of demersal predators than other assemblages, primarily medium winter skate and flatfish (Fig. 8A). The MGB piscivores were dominated by large winter skate (Table 3), in

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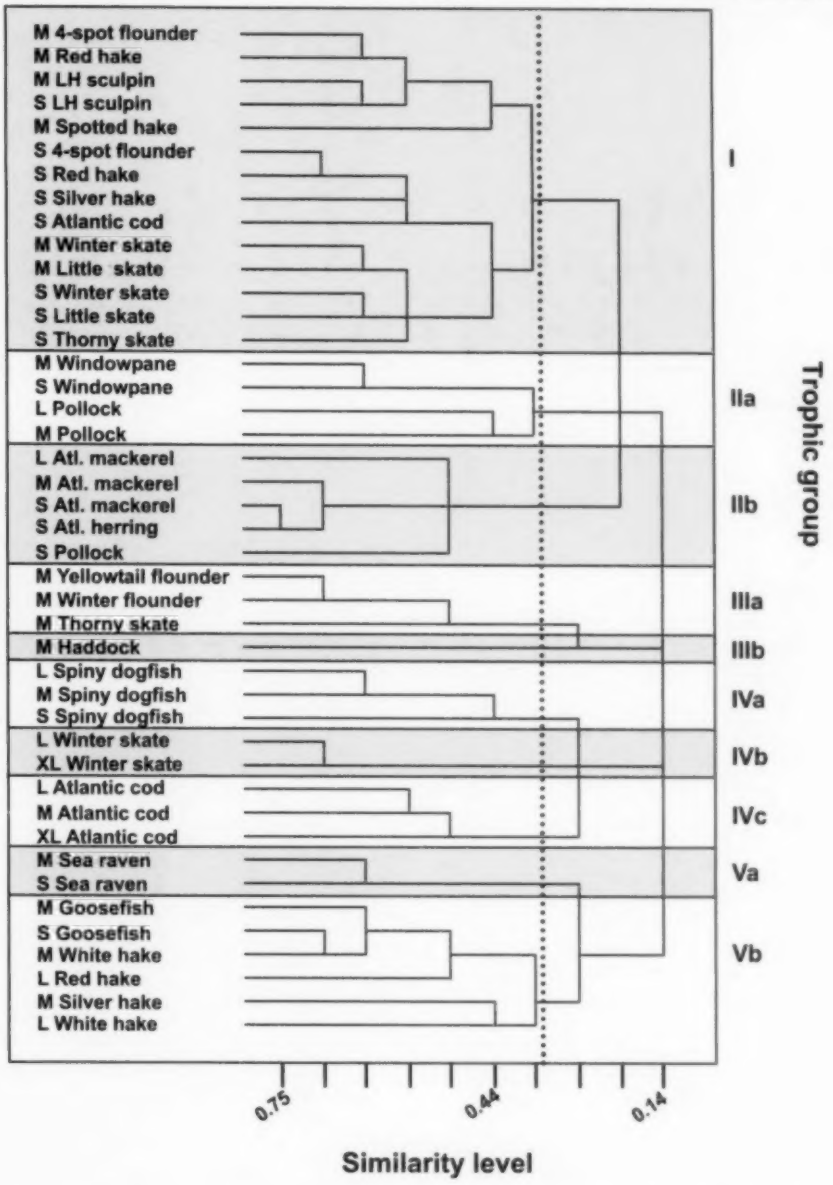
Fig. 6. Dendrogram of diet similarity (Schoener's index) during spring. Trophic groups are identified at similarity levels >0.34.



contrast with the other assemblages. The NFS assemblage also had a high proportion of piscivores, again reflecting high spiny dogfish biomass (Table 2), and it also had high biomass of planktivores (primarily Atlantic herring, Table 2) and demersal predators (primarily haddock, Table 2; Fig. 8A).

During spring, patterns in spatial and trophic resource use were different from those during autumn, and these generally reflected seasonal changes in spatial distribution and diets of dominant species. Primarily, the generalist trophic guild replaced the piscivores as the dominant group and became the most important biomass component in both the SNE and OFF assemblages (Fig. 8B). This reflects both the seasonal shift away from piscivory (Figs. 4 and 6) and a southward and offshore migration of medium and large spiny dogfish (Table 3). The planktivores, primarily Atlantic herring (Table 3), were also important in the SNE assemblage during spring. In addition to the generalist spiny dogfish, the OFF assemblage was dominated by shrimp/amphipod predators (medium silver hake, Table 3) and the planktivores including Atlantic herring, Atlantic mackerel, and butterfish (Fig. 8B; Table 3). The spring MGB assemblage was also dominated by generalists, and these included large winter skate (total 16.5%) and Atlantic cod (total 10%) in addition to medium and large spiny dogfish (total 21.3%)

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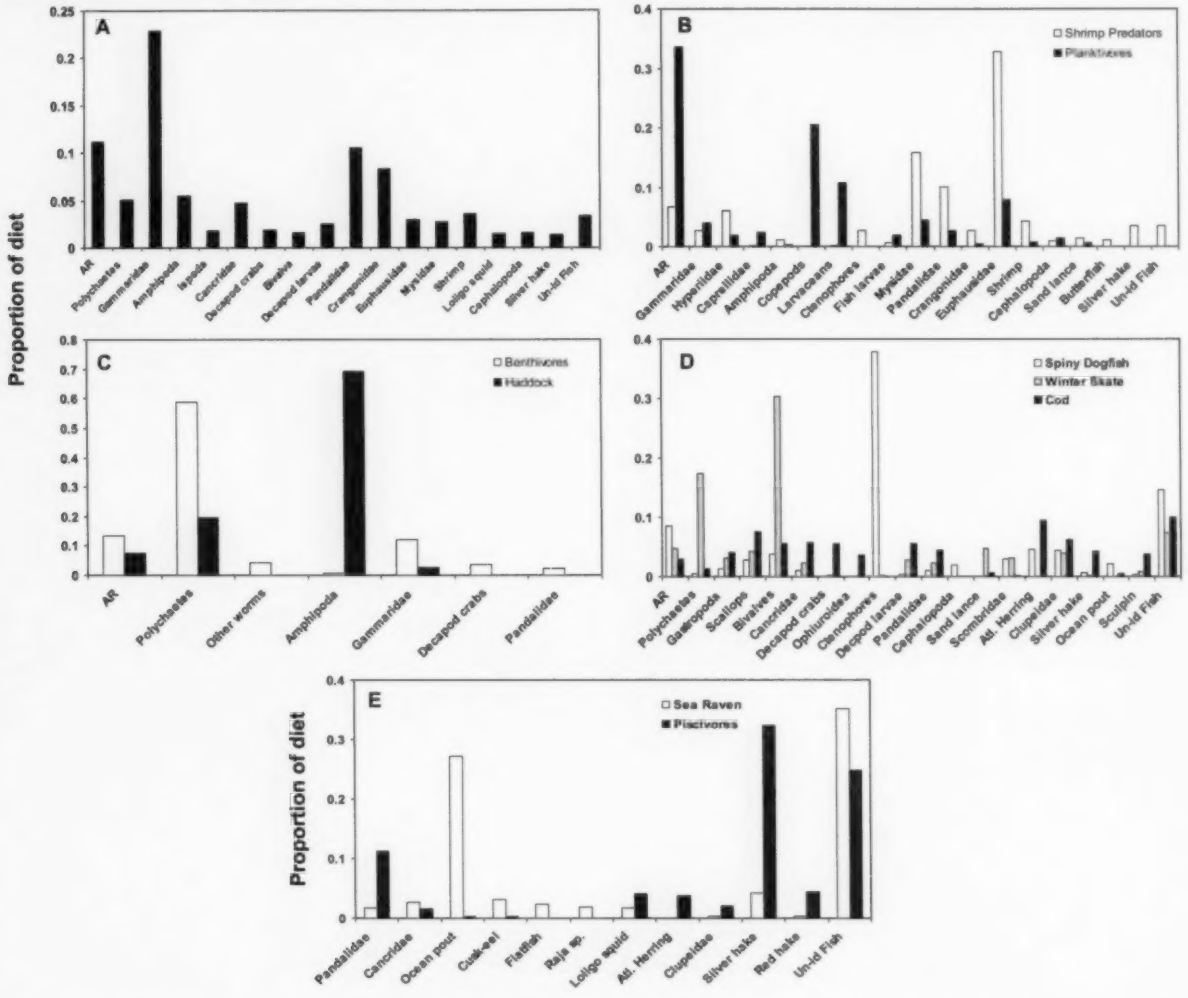
Fig. 7. Mean diet composition of five major trophic groups (Fig. 6) during spring: (A) shrimp/amphipod predators, (B) pelagic predators, (C) benthivores, (D) generalists, and (E) piscivores.



(Table 3). Both shrimp/amphipod feeders (medium winter and little skate, total 17.7%) and demersal predators (haddock, total 13%) were more important on MGB than in southern and deeper habitats (Fig. 8B). The spring NFS assemblage also included a diverse suite of predators, again dominated primarily by spiny dogfish (35%) but included Atlantic cod (9.2%) (Table 3). The pelagic predators were primarily the shrimp predator pollock (13%), while the demersal predators were represented by haddock (7.8%) (Table 3; Fig. 8B).

Discussion

Spatial structure on Georges Bank

Depth gradients strongly influence the spatial distribution of fish taxa on Georges Bank and result in species co-distribution and geographic assemblages. In addition, there is a latitudinal gradient in species composition, reflecting both temperature gradients and the broader geographic distribution of component species. These spatial patterns are consistent with broad-scale regional assemblages in the northwest Atlantic (Gabriel 1992).

Despite the major changes in species composition, the boundaries of geographic assemblages on Georges Bank are remarkably similar to those observed in autumn during the period from 1963 to 1978 (Overholtz and Tyler 1985). However, changes in species abundances and spatial distributions through time have resulted in shifts in species composition within assemblages. For example, haddock was a numerical dominant in four of five assemblages in the analysis of Overholtz and Tyler (1985), but it is only present in the autumn NFS assemblage in the current study. In contrast, spiny dogfish was never a numerical dominant in any assemblage during the earlier time period (Overholtz and Tyler 1985), but it dominates the biomass of most assemblages during both seasons in the current study. These changes in spatial

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Table 4. Partial Mantel regression and path analysis.

Regression	Z	SP	β	r	Path
Fall surveys					
Depth (**Z**) × space (**S**)	39.42	13.71	0.37	0.58***	
Z × diet (**D**)	62.69	2.18	0.06	0.08 ns	Depth
S × **D**	25.14	0.22	0.01	0.01 ns	↘
(**Z** × **S**)|**D**	13.70	13.70	0.88	0.58***	Space
(**Z** × **D**)|**S**	1.99	1.99	0.08	0.08 ns	Diet
(**S** × **D**)|**Z**	0.62	0.62	0.06	0.04 ns	
Spring surveys					
Z × **S**	30.89	11.52	0.32	0.57***	
Z × **D**	49.85	1.47	0.04	0.05 ns	Depth
S × **D**	17.59	0.38	0.03	0.02 ns	↘
(**Z** × **S**)|**D**	11.50	11.50	1.0	0.57***	Space
(**Z** × **D**)|**S**	1.08	1.08	0.04	0.04 ns	Diet
(**S** × **D**)|**Z**	0.09	0.09	0.01	0.01 ns	

Note: Forty-six predators were included in the autumn analysis, and 43 predators were included in the spring analysis. Z, Mantel correlation coefficient; SP, sum of products; β, regression parameter; r, standardized Mantel coefficient. Statistical significance of Mantel coefficients tested by permutation tests (5000 permutations, Mantel 1967) at the Bonferroni-corrected $\alpha = 0.05/6 = 0.0083$. ***$p < 0.001$; ns, $p > 0.01$.

distribution are related to changes in population sizes. As haddock populations have declined through time, they have become more concentrated on the northeast peak of Georges Bank. Conversely, the general increase in spiny dogfish populations over the last three decades has been accompanied by an expansion of ranges (Garrison and Link 2000*b*). This is consistent with patterns observed in other continental shelf fish assemblages where the geographic area covered by a stock is related to the stock size (Atkinson et al. 1997). Changes in spatial distribution may also be associated with generally warmer bottom temperatures in the northwest Atlantic during the 1990s relative to the 1960s (Holzwarth and Mountain 1990).

The geographic boundaries of spatial assemblages on Georges Bank are also consistent over seasonal time scales. The only major seasonal difference is the incorporation of the autumn southern flank assemblage into the spring southern New England assemblage. However, there were major seasonal changes in species composition within assemblages, reflecting the migration of dominant species.

During autumn, medium and large spiny dogfish were absent from the offshore assemblage and were by far the biomass dominants in the SNE and NFB assemblages, accounting for 78 and 51% of the total biomass, respectively. During spring, spiny dogfish migrated south and offshore. They remained dominant in SNE and became the biomass dominant in the OFF assemblage, accounting for 71% of the total biomass. Given the extremely high biomass of this species and their predatory nature, these patterns impart a significant seasonal change in the predator field within these geographic regions.

Seasonal migrations also impact the prey field within spatial assemblages. During autumn, Atlantic herring is present only in the northern assemblages, while during spring, Atlantic herring expands its range south and becomes important in the SNE and OFF assemblages. Longfin squid and butterfish are also only seasonally abundant in the Georges Bank region. They are broadly distributed across Georges Bank and southern New England during autumn and dominate the biomass of the offshore and southern flank assemblages. During spring, these species migrate to deeper habitats beyond the range of the survey and are present only in the offshore assemblage. Seasonal migrations have a significant impact on the trophic structure within assemblages.

Trophic structure on Georges Bank

The major trophic groups (piscivores, demersal predators, shrimp predators, etc.) identified in the Georges Bank fish community reflect broad patterns in prey preferences. Members of these groups consume similar prey at broad taxonomic levels (e.g., fish) or within a similar habitat type (e.g., demersal predators feed upon a variety of benthic invertebrates). These major groups reflect gradients in predator feeding and habitat. In general, there is segregation among the fish community between predators consuming small versus large prey (e.g., invertebrates versus fish) and benthic versus pelagic prey (e.g., polychaetes versus euphausiids). These gradients are consistent with both patterns observed at larger scales within the northwest Atlantic (Garrison and Link 2000*a*) and patterns of resource utilization for fish communities in general (Ross 1986).

At a lower hierarchical level, subgroups cluster together at similarity levels greater than 40% during autumn and 34% during spring. These reflect resource partitioning at a more detailed taxonomic level. For example, within the piscivores during autumn, predators with a majority of their diet comprising Atlantic herring are distinguished from the major piscivores that consume a broader suite of prey. The similarity levels that define these groups are comparable with those observed both at a broader spatial scale including the entire northeast U.S. continental shelf (Garrison and Link 2000*a*) and in different time periods on Georges Bank (Garrison and Link 2000*b*).

There are significant seasonal changes in the trophic structure in this community associated with both predator and prey movements. The most striking seasonal difference is the addition of the generalist groups during spring. During autumn, the members of this group were piscivores (large

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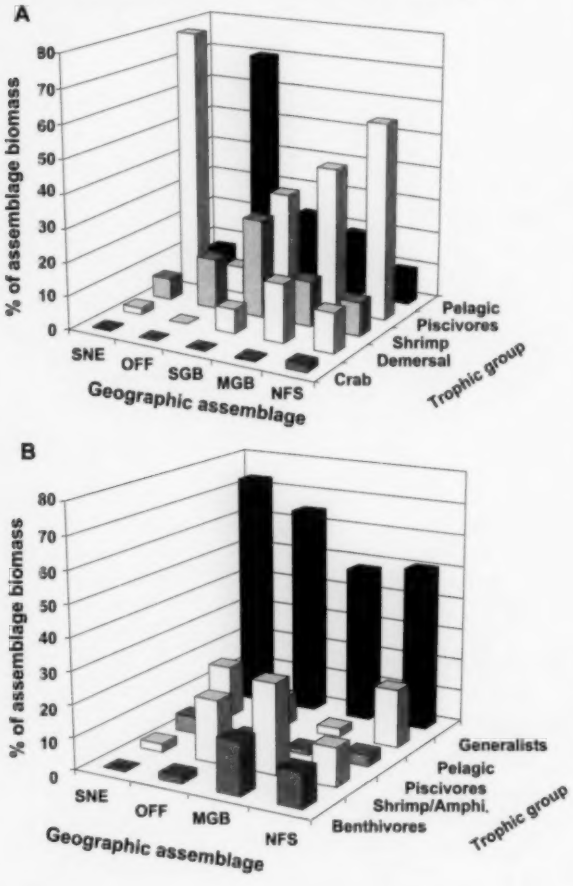
Fig. 8. Proportion of total biomass in each spatial assemblage by major trophic group during (A) autumn and (B) spring.



and extra large winter skate, large and medium spiny dogfish, large Atlantic cod), crab predators (extra large Atlantic cod), shrimp predators (medium Atlantic cod), or planktivores (small spiny dogfish). The most notable change in the diets of the piscivores is a decline in the importance of fish prey. For example, large Atlantic cod diets were 67% fish prey during the autumn and only 24% during the spring. Large winter skate diets were 35% fish in the autumn and 16% in the spring. Atlantic herring was the most important prey item for the major piscivores aside from unidentified fish during autumn. However, in the spring, silver hake became the major prey. These seasonal changes in piscivore diets are related to seasonal movements of major prey. For example, Atlantic herring undergo seasonal migrations as noted above.

With reduced availability of fish prey, the large predators in this system incorporate a broader range of prey into their diets. Atlantic cod, for example, has a very broad diet during the spring including both benthic and pelagic prey of all sizes. Predator movements have similar results, e.g., increases in the importance of ctenophores in large and medium spiny dogfish diets are associated with their southern and offshore migration during spring. Most predators in this system exhibit highly plastic diets that appear to be strongly related to patterns in prey availability.

Ontogenetic diet shifts are also a major feature of the trophic structure in this community. The piscivores in this system, including spiny dogfish, silver hake, Atlantic cod, winter skate, and white hake, are piscivorous only in larger size-classes, while they prey upon shrimp-like taxa or small benthic invertebrates in small sizes. Size-based differences in diets are less pronounced in predators feeding at lower trophic levels (i.e., flatfish), and there are several important predators (e.g., goosefish and sea raven) that are piscivorous in all sizes. The ontogenetic shifts in diet are generally not related to major changes in habitat; all size-classes of most of these species are found in the same assemblage. Rather, these shifts are likely related to changes in body size and predator morphology. Shifts in diets with body size are a frequent occurrence in fish systems, particularly in piscivorous species (Werner and Gilliam 1984). Given that the species showing strong ontogenetic diet shifts are also biomass dominants, the changes in the functional role of these species through life history are an important component of the trophic dynamics of this system.

Resource partitioning on Georges Bank

Fish communities typically partition resources across spatial, temporal, and trophic dimensions (Ross 1986). Differing patterns of resource use along multiple dimensions may reflect the influence of competition in structuring species assemblages (Schoener 1974; Pianka 1980; Ross 1986). Where competition is important (i.e., resources limit populations), species with high overlap in resource use along one dimension (e.g., high dietary overlap) should have a low overlap in resource use along a second dimension (e.g., low spatial overlap) to avoid the negative effects of interactions (Schoener 1974; Pianka 1980). Evidence of such "niche complimentarity" suggests the importance of competition (Ross 1986). In assemblages where species are from a broad range of taxonomic histories, overlap should vary with resource availability and the abundance of potential competitors (Ross 1986). Under these conditions, competition is an active mechanism influencing spatial distributions, species interactions, and population dynamics of component species. Such active shifts in resource overlap under varying resource conditions were recently demonstrated by Piet et al. (1999) in an assemblage of tropical fish.

Although depth gradients are important in determining the spatial overlap between species on Georges Bank, the Mantel regression analysis demonstrates that dietary overlap is independent of spatial patterns. If competition were a strong factor influencing spatial and trophic structure, then we would expect a strong negative relationship between spatial and dietary overlap. Direct competition for food resources has been hypothesized as a cause for apparent indirect effects of overexploitation in this system, specifically the increase in elasmobranchs during the decline in gadid and flatfish populations (Fogarty and Murawski 1998). Dietary overlap has been cited as evidence for a potential competitive interaction between these groups (Fogarty and Murawski 1998). The more detailed analysis in the current study does not support this hypothesis and suggests that

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strong exploitative competition is not an important factor driving spatial and trophic structure on Georges Bank.

Community structure and ecosystem management

The strong spatial differences in trophic structure on Georges Bank reflect differences in species membership and biomass dominance among the spatial assemblages. Associated with these patterns are differences in the complexity of food webs, the strength of species interactions, and the resulting energy and biomass flow in this system. For example, single trophic groups dominate the offshore and southern New England assemblages in the fall, whereas the trophic complexity on the southern flank and main portion of Georges Bank is higher and includes a more diverse group of species at multiple trophic levels. Along with these spatial gradients in community structure, there is strong seasonal variation in both predator and prey spatial distributions and the resulting interactions between species. While traditional fisheries models typically aggregate single species stocks at regional or larger scales, the current analysis demonstrates that the ecological structure within this community varies at the subregional level. The spatial assemblages on Georges Bank may be considered ecologically distinct functional units within the larger community. Ecosystem approaches to fisheries assessment and management should explicitly account for these spatial and temporal differences in community structure.

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Interrenal metallothionein and cortisol secretion in relation to Cd, Cu, and Zn exposure in yellow perch, *Perca flavescens*, from Abitibi lakes

J.-S. Laflamme, Y. Couillard, P.G.C. Campbell, and A. Hontela

Abstract: The concentrations of Zn, Cu, and Cd in the interrenal tissue and liver of yellow perch, *Perca flavescens*, sampled in six lakes in the mining region of Abitibi (Quebec) revealed a gradient of contamination: reference lakes, intermediate-contaminated lakes, and highly contaminated lakes. Interrenal and hepatic metallothionein concentrations followed a similar gradient, and there was a strong relationship between metallothionein and total metal (Zn + Cu + Cd) content in the liver and Cu + Cd content in the interrenals. Following a standardized confinement stress test, plasma cortisol and glucose were significantly higher in fish from reference lakes compared with the two most contaminated lakes. No differences in plasma chloride were observed. The secretory response of the interrenal tissue to in vitro stimulation by adrenocorticotropic hormone and dibutyryl cyclic AMP was significantly lower in fish from a contaminated lake compared with fish from a reference lake. Condition factor was significantly lower in yellow perch from the most contaminated lakes. This study demonstrated that a chronic field exposure to base metals increases hepatic and interrenal tissue metallothionein concentrations and disrupts the physiological capacity of yellow perch to generate the normal hormonal stress response.

Résumé : Les concentrations du Zn, Cu et Cd dans le tissu interrénal et le foie de la perchaude, *Perca flavescens*, échantillonnée dans six lacs de la région minière de l'Abitibi (Québec) ont démontré un gradient de contamination : lacs référence, lacs à contamination moyenne et lacs à contamination forte. Les concentrations de la métallothioneine interrénale et hépatique ont suivi un gradient similaire, avec une corrélation entre métallothioneine et les métaux totaux (Zn + Cu + Cd) dans le foie et le Cu + CD dans les interrénales. Suite à un test de stress standardisé, le cortisol et glucose plasmatique étaient plus élevés chez les poissons des lacs référence, comparé aux lacs le plus contaminés, tandis que le chlorure plasmatique n'était pas différent. La réponse secrétrice du tissu interrénal à une stimulation in vitro par l'hormone adrénocorticotropique and le dibutyryl AMP cyclique, ainsi que le facteur de condition, était plus faibles chez les poissons des lacs contaminés comparé aux lacs référence. L'étude a démontré que l'exposition chronique sur le terrain à des métaux augmente les concentrations de la métallothioneine et perturbe la capacité physiologique normale de générer la réponse hormonale au stress chez la perchaude.

Introduction

The hypothalamo–pituitary–interrenal axis of teleost fish is activated by abiotic and biotic stressors (Barton and Iwama 1991; Hontela 1997) that promote the release of hypothalamic corticotropin-releasing hormone and pituitary adrenocorticotropic hormone (ACTH) to stimulate the synthesis and release of cortisol by the interrenal tissue located in the pronephros (Wendelaar Bonga 1997). Cortisol has effects on intermediary metabolism (Van Der Boon et al. 1991), osmoregulation (Laurent and Perry 1990), the reproductive system (Pankhurst and Van Der Kraak 2000), and the immune system (Pickering 1989). The stress response in fish, characterized by elevated plasma cortisol, favours, together with other hormones, recovery of homeostasis and its maintenance (Barton and Iwama 1991; Wendelaar Bonga 1997).

Numerous laboratory studies have reported increased plasma cortisol levels following acute exposure of fish to environmental pollutants, including metals (reviewed in Hontela 1997). In contrast, studies of chronic exposure effects on cortisol levels and the interrenal function are scarce. Chronic (180 days) laboratory exposure to Hg reduced cortisol levels in walking catfish, *Clarias batrachus* (Kirubagaran and Joy 1991), and juvenile walleye, *Stizostedion vitreum* (Friedmann et al. 1996). Lower cortisol response following a stress has been documented in northern pike, *Esox lucius*, and yellow perch, *Perca flavescens*, sampled at sites contaminated by metals (Lockhart et al. 1972; Hontela et al. 1992; Brodeur et al. 1997*a*). Recent in vitro studies in our laboratory demonstrated that short-term (60 min) exposure of interrenal cells to Cd, Hg, or Zn impairs cortisol se-

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J.-S. Laflamme and A. Hontela.[1] Département des Sciences Biologiques, TOXEN Research Centre, Université du Québec à Montréal, C.P. 8888, Succ. Centre-Ville, Montréal, QC H3C 3P8, Canada.
Y. Couillard. Département des Sciences Biologiques, Université de Montréal, C.P. 6128, Succ. A, Montréal, QC H3C 3J7, Canada.
P.G.C. Campbell. INRS-Eau, Université du Québec, C.P. 7500, Sainte-Foy, QC G1V 4C7, Canada.

[1]Author to whom all correspondence should be addressed.
e-mail: hontela.alice@uqam.ca

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cretion at doses that are not cytotoxic (Leblond and Hontela 1999). However, the mechanisms through which chronic environmental exposures to base metals disrupt cortisol secretion in fish have not been elucidated thus far.

In the present field study, we investigated some of the cause–effect relationships between contamination by metals and interrenal dysfunction in yellow perch by sampling fish in lakes representing a gradient of metal concentrations. The study was designed to determine if dose–response relationships can be established between the in situ exposure to metals, the tissue load (burden), and effects on the physiological and endocrine status of the fish. Metal concentrations were measured in the liver, an important site of metal accumulation, in order to rank selected sites according to hepatic contamination of the indigenous yellow perch. For the first time in teleost fish, metals were also measured in the interrenal tissue to test the link between exposure and metal levels in this endocrine tissue. To assess the capacity of the interrenal tissue to resist metal toxicity, metallothionein (MT), a detoxification protein, was measured in both the liver and the interrenals. MT is a low molecular mass ubiquitous protein, rich in cysteine, and its synthesis is mainly induced by metals. It plays a key role in the detoxification of base metals and the regulation of essential metals such as Cu and Zn (Roesijadi 1992). To test the link between tissue load of metals and effect on the endocrine status, the capacity of the interrenal tissue to generate the normal cortisol stress response was assessed: first in vivo by measuring plasma cortisol in yellow perch subjected to a 1-h confinement stress and then in vitro by measuring cortisol output in response to ACTH and, for the first time in a wild fish, to dibutyryl cyclic AMP (dbcAMP), a second messenger mediating the effect of ACTH (Patiño et al. 1986). Because cortisol has a role in intermediary metabolism, the link between tissue load and effects was also tested by measuring the condition factor of all the fish. Thus, the objectives of this study were to (*i*) characterize the exposure of yellow perch from lakes in a mining region, (*ii*) test the link between base metal and MT levels in liver and interrenal tissue, and (*iii*) test the link between exposure, the capacity to generate the normal cortisol response, and effects on growth (condition).

Materials and methods

Study sites and fish species

Fish were sampled in six lakes (Lakes Opasatica, Dasserat, Vaudray, Bousquet, Dufualt, and Osisko) of the mining area of Rouyn-Noranda, located in northwestern Quebec, Canada (48°00′N, 79°00′W), in June 1997. Lakes in the area are affected by acid and metal contamination from current metal mining operations, abandoned mine sites, and atmospheric deposition from a nearby smelter (Couillard et al. 1993). Previous studies (Couillard et al. 1993; Tessier et al. 1993; Brodeur et al. 1997*a*) provided data on metal concentrations in water, sediments, and yellow perch livers and enabled us to make a preliminary ranking of the lakes based on contamination: Lakes Opasatica and Dasserat as reference sites, Lakes Vaudray and Bousquet as intermediate-contaminated sites, and Lakes Osisko and Dufault as the most highly contaminated lakes. Table 1 shows concentrations in water and sediments from a more recent sampling. For a detailed map of the area and limnological characteristics of the lakes, see Couillard et al. (1993) and Brodeur et al. (1997*a*).

Yellow perch was used for this study because it is a relatively sedentary fish (Aalto and Newsome 1990) that reflects the contamination of the site where it is sampled, and it is an abundant species in the six sampled lakes. Males and females were sampled; fish were sexually immature (spawning occurs in May). The fish were collected in the postspawning period to minimize variation of the cortisol response due to differences in gonadal status (Pottinger et al. 1995).

Experimental treatments

Fish were captured by rod, seine, or gill net, since capture method does not influence plasma cortisol in fish kept in in situ enclosures following capture and blood sampled the next day (Brodeur et al. 1997*b*). Fish were placed into floating enclosures (0.5 m wide × 1 m long × 1 m deep) made of net (10 fish per enclosure) for at least 16 h so they could partially recover from the stress of capture. They were not fed during this period. Previous studies have shown that a 15- to 24-h rest period in enclosures postcapture facilitates recovery from the initial stress of capture, as indicated by significantly lower plasma cortisol levels compared with values immediately postcapture (Brodeur et al. 1997*a*, 1997*b*; Girard et al. 1998).

Standardized in vivo stress test

The day after capture, fish were taken from the enclosures and placed into 20-L containers by groups of 10 for 1 h. The 1-h confinement stress used in the present study following the period of recovery significantly elevated cortisol levels in comparison with fish that were not confined. However, the stress was not too severe, since morbidity or mortality of yellow perch was not observed during the confinement period. The confinement stress test always began at 10:00, and each additional group was put into different containers 15 min later. The fish were stressed during the same period of the day to avoid diel effects on cortisol secretion (Audet and Claireaux 1992). After 1 h of confinement stress, fish were anaesthetized by adding 150 mg tricaine methanesulfonate (MS 222)·L^{-1} to the container. Yellow perch were bled from the caudal vasculature with a 1-mL heparinized syringe. Another group of 12 fish·site^{-1}·day^{-1} were removed from the enclosures and at 10:00 were anaesthetized directly in the MS 222 solution: these fish are referred to as the "unstressed group." A maximum of 40 fish·day^{-1} were sampled, and about 10 min was necessary to bleed each group of 10 fish. Bleeding of all the fish was completed within 1 h. Fish and syringes were put on ice in a cooler and transported to a field laboratory; sites were located 20–45 min from the laboratory. The time between the end of blood sampling at the site and the beginning of dissection in the laboratory was kept the same (45 min) for all the lakes.

At the field laboratory, blood was centrifuged (5 min at 10 000 × *g*) and plasma was frozen at –20°C for later analyses. Body mass and length of fish were recorded for the calculation of condition factor (weight (grams)/(length (centimetres))^3 × 100). Liver and interrenal tissue were dissected for metals and MT analyses and immediately frozen and kept in liquid nitrogen to avoid oxidation. Upon return to the university facilities (1 month later), tissue samples were transfered to a –80°C freezer and maintained in oxygen-free sealed bags. Hepatosomatic index was calculated as liver weight (grams)/body weight (grams) × 100.

In vitro ACTH and dbcAMP challenge

The functional integrity of the interrenal tissue was tested in vitro in yellow perch from a reference lake (Lake Opasatica) and a contaminated lake (Lake Osisko). The day after capture, a group of 12 fish·site^{-1}·day^{-1} were removed from the enclosures and at 10:00

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Table 1. Trace metal concentrations (mean ± SE) in water, sediments, and yellow perch sampled in previous studies in the Rouyn-Noranda region.

	Free metal ion in the dissolved phase (nmol·L^{-1})				Metals in oxic sediments (nmol·g dry weight^{-1})					Metals in yellow perch liver (nmol·g wet weight^{-1})[b]			
Lake	Year	N	[Zn^{2+}]	[Cu^{2+}]	[Cd^{2+}]	Year	N	Extract {Zn}	Extract {Cu}	Total {Cd}	Year	N	Zn	Cu	Cd
Opasatica	1998	8	19.3±3.0	3.4±0.7	0.12±0.06	1997	4	105±20	144±25	5.1±0.7	1996	20	612±21	153±25	28±7
	1989				0.36[a]	1989				3.76[a]					
Dasserat											1996	20	474±35	121±19	17±2
Bousquet	1998	6	39.0±2.0	2.9±0.4	0.40±0.01	1997	3	204±44	143±9	10.0±0.1					
	1989				2.32[a]	1989				30.1[a]					
Vaudray	1998	6	35.0±1.0	8.9±4.6	0.46±0.02	1997	1	320	238	12					
						1989				38.3[a]					
Osisko	1997	1	20	2.2	0.47	1997	1	68 500	105 000	590	1996	20	765±31	425±44	125±7
Dufault	1998	4	250±20	9.6±0.8	1.65±0.14	1997	1	18 000	19 400	200	1996	20	749±90	299±36	205±16

Note: Free metal ion concentrations were directly measured in aqueous media using dialysers installed at 10 cm above the sediments and an ion-exchange technique; see Fortin and Campbell (1998) for details on the ion-exchange technique. [M^{2+}] were estimated from sediment–water sorptive equilibria; see Tessier et al. (1993) for details. {M} were extracted for 6 h at 96°C with 0.04 M $NH_2OH{\cdot}HCl$ in 25% (v/v) HOAc and the residue was extracted at 85°C for 5 h with 30% H_2O_2 adjusted to pH 2 and then at room temperature with 3.2 M NH_4Oac in 20% (v/v) HNO_3.

[a]Data from Tessier et al. (1993).

[b]Data from Brodeur et al. (1997*a*).

were anaesthesized directly in MS 222 solution ("no-stress groups"). Fish were bled and transported to the field laboratory.

The microplate method has been described elsewhere (Benguira and Hontela 2000). In summary, each head kidney was dissected, cut into small fragments (1 mm^3), and divided into three equal parts. Each part was put into a microplate well containing 1 mL of nutritive medium (MEM). After 2 h of incubation necessary to reach the basal rate of cortisol secretion, the microplate was centrifuged and the supernatant removed and each of the three wells received a different treatment: stimulation by 1 mL of MEM containing 2 IU of porcine ACTH (Sigma Chemical Co., St. Louis, Mo.), stimulation by 1 mL of MEM containing 4 mmol of dbcAMP (Sigma Chemical Co.), both optimal doses determined in a pilot study, and incubation in 1 mL of MEM only. After 3 h of incubation, each well was centrifuged and the supernatant frozen for later analyses of cortisol by radioimmunoassay.

Analyses of metals and MT

Metals and MT levels were measured in the liver and the interrenal tissue from fish that were subjected to the in vivo stress test. Concentrations of Cd, Cu, and Zn in both tissues were measured by flame atomic absorption spectrometry using tissue homogenates corresponding to 100 mg dry weight. Cytosolic MT concentrations were measured by a Hg saturation assay adapted from Dutton et al. (1993) and Couillard et al. (1993). Fresh bovine hemoglobin was used as the exogenous protein for removal of excess non-MT-bound Hg. Homogenates of both tissues were prepared from combined samples from at least three randomly chosen fish from the same lake to obtain sufficient dry weight for both analyses.

The summation of the metals in each combined sample ([Zn + Cu + Cd]), in order to establish a relationship with MT concentrations, was calculated as

$$(1)\qquad [Zn + Cu + Cd] = [Zn]/K_{Zn}AM_{Zn} + [Cu]/K_{Cu}AM_{Cu} + [Cd]/K_{Cd}AM_{Cd}$$

where [Zn], [Cu], and [Cd] are the concentrations of the metals in the combined samples (micrograms per gram dry weight), K is the specific capacity of each metal to bind to MT by metal thiolate linkages (seven equivalents for bivalent metals (Zn and Cd) and 12 equivalents for univalent metals (Cu), as reported by Kägi and Schäffer (1988), and AM is the atomic mass of each metal. To calculate Cu and Cd summation ([Cu + Cd]) in combined samples of the interrenal tissue, the same calculation was done as eq. 1, except that [Zn] was not added.

Analyses of blood parameters

Concentrations of cortisol in the plasma and the incubation medium from the microplates were measured with a radioimmunoassay kit (ICN Biomedicals Canada Ltd., Montreal, Que.). Plasma chloride and glucose levels were determined using a colorimetric reagent method (Sigma Chemical Co.).

Statistical analyses

For all the tests, we used a statistical significance level of $\alpha = 0.05$. Since no difference between sexes was detected within each site (t test, $P < 0.05$), the two sexes were grouped for analysis. Differences amoung groups (except for in vitro results) were tested using one-way analysis of variance (ANOVA, SAS 6.12 program). The Tukey–Kramer test was used to differentiate different means. Data were transformed, when necessary, to obtain normality and homoscedasticity. Relationships between concentrations of metals and MT were evaluated with Pearson's correlation coefficient. Data were ln transformed to obtain homogeneity and normality of the residue values. Bonferroni's correction of the statistical signifi-

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cance level was used. Analysis of covariance (ANCOVA) was used for the in vitro method because final cortisol secretion correlated with basal cortisol secretion before stimulation (Pearson's test, $r = 0.74$, $P < 0.001$). Final cortisol secretion was used as the dependent variable, basal cortisol secretion as the covariate, and lake and treatment as the independent variables. Data were ln transformed to obtain homogeneity. The model used explained 86.9% of the variability.

Results

Characteristics of sampled yellow perch

Yellow perch from Lake Opasatica were larger (greater length and weigth) and those from Lake Dufault were smaller compared with fish from all the other lakes (Table 2), which were not different from each other in length and weight. Condition factor was greater in yellow perch from reference (Lakes Opasatica and Dasserat) and intermediate-contamination lakes (Lakes Bousquet and Vaudray) than in those from high-contamination lakes (Lakes Osisko and Dufault) (Table 2). The hepatosomatic indices of fish collected in Lakes Dasserat and Vaudray were higher than in the other lakes, all of which were similar (except Lake Dufault < Lake Opasatica).

Metals and MT concentrations

The levels of Zn, Cu, and Cd were higher in pooled samples of liver and interrenal tissue of yellow perch from the most contaminated lakes (Lakes Osisko and Dufault) compared with the reference and intermediate-contamination lakes (Table 3). Liver and interrenal concentrations of Cd were higher in intermediate-contamination lakes (Vaudray and Bousquet) than in reference lakes (Lakes Opasatica and Dasserat). Concentrations of Cu and Cd were consistenly higher in the liver, compared with the interrenal tissue, in yellow perch within the same lake.

Tissue MT concentrations in pooled samples of liver or interrenal tissue of yellow perch increased progressively from the least to the most contaminated lakes: fish from the two reference sites (Lakes Opasatica and Dasserat) had the lowest MT concentrations, and fish from the two most contaminated lakes (Lakes Osisko and Dufault) had the highest levels (Fig. 1). Similarly to the concentrations of metals, MT levels were higher in liver compared with the interrenal tissue in yellow perch within the same lake. MT concentrations correlated well with the concentrations of each metal (Zn, Cu, and Cd) in both tissues (Table 3). In the liver, MT levels highly correlated in a linear way with the sum of the metals, [Zn + Cu +Zn] (Fig. 2*a*). In the interrenal tissue, MT concentrations highly correlated in a linear way with the sum of Cu and Cd, [Cu + Cd] (Fig. 2*c*), but the relationship between MT levels and the summation of the three metals seemed to reach a plateau (Fig. 2*b*).

Cortisol response

Following a standardized stress test, plasma cortisol levels were generally higher in sampled adult yellow perch from reference lakes (Lakes Opasatica and Dasserat) and an intermediate lake (Lake Vaudray) compared with the most contaminated lakes (Lakes Osisko and Dufault) (Fig. 3). Fish from Lake Bousquet (intermediate) had higher plasma cortisol levels than fish from Lake Dufault but not fish from Lake Osisko. In vitro cortisol secretion by the interrenal tissue in response to 2 IU of porcine ACTH or 4 mmol of dbcAMP stimulation was higher in interrenal tissue from the reference lake (Lake Opasatica) compared with Lake Osisko, the highly contaminated lake (Fig. 4). Within each lake, no difference in the cortisol secretion was observed between the ACTH and dbcAMP stimulated responses. No difference was detected for the unstimulated interrenal tissue secretion between the two lakes (Fig. 4).

Blood parameters before and after a standardized stress test

Plasma chloride, glucose, and cortisol levels were compared (Table 4; Fig. 3) between a yellow perch group subjected to a standardized confinement stress test and a group sampled without the stress test from three lakes: Lake Opasatica (reference), Lake Vaudray (intermediate contamination), and Lake Osisko (high contamination). Plasma cortisol levels were higher in the group after the standardized stress test compared with the unstressed group for each lake (means from unstressed fish ranged between 82.9 and 118.9 $ng \cdot mL^{-1}$ and were not significantly different between the three lakes; data not shown). Plasma chloride levels within each type of stress status group were not different between the lakes. Stressed yellow perch from Lakes Opasatica and Osisko, but not Lake Vaudray, had lower plasma chloride levels compared with unstressed fish from the same lake. Stressed fish from the highly contaminated lake had lower glucose levels than stressed fish from the less contaminated lake. Plasma glucose levels of the stressed fish were higher in Lakes Opasatica and Vaudray compared with those of the unstressed fish from the same lake. No difference in plasma glucose levels between stressed and unstressed yellow perch groups from the highly contaminated lake (Lake Osisko) was observed. (Table 4).

Discussion

Exposure of yellow perch was characterized by measuring metals in the liver and the interrenal tissue. Concentrations of Zn, Cu, and Cd in the liver of wild yellow perch sampled in the present study increased progressively from the least to the most contaminated lake: the six lakes sampled thus provide an excellent in situ system for investigating the effects of metal contamination on the physiology of resident yellow perch. Levels of Cu and Cd in the liver of yellow perch from our reference lakes were similar to those reported for wild European perch, *Perca fluviatilis*, from reference sites by Olsson and Haux (1986). Concentrations of Cd in the liver of yellow perch from the two most contaminated lakes (Lakes Osisko and Dufault) ranged from 41 to 61 $\mu g \cdot g$ dry $weight^{-1}$. These values were higher than mean hepatic Cd levels from the most contaminated sites in various in situ studies with either European perch (6–31 $\mu g \cdot g$ liver dry $weight^{-1}$; Olsson and Haux 1986; Hogstrand et al. 1991) or salmonids (2–19 $\mu g \cdot g$ liver dry $weight^{-1}$; Roch et al. 1982; Farag et al. 1995).

This was the first study to characterize metal concentrations in the interrenal tissue of wild fish. Similar to Zn, Cu, and Cd levels in the liver, concentrations of metals in the

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Table 2. Characteristics (mean ± SE) of adult yellow perch collected in six lakes of the Rouyn-Noranda mining region.

	N	Length (cm)	Weight (g)	Hepatosomatic index (%)	Condition factor
Reference lakes					
Opasatica	31	21.1±0.6 *a*	100.1±7.8 *a*	1.20±0.05 *b*	1.13±0.02 *ab*
Dasserat	63	17.6±3.3 *b*	67.1±3.9 *b*	1.44±0.02 *a*	1.15±0.01 *a*
Intermediate-contamination lakes					
Bousquet	32	17.7±0.5 *b*	70.0±6.1 *b*	1.07±0.02 *bc*	1.16±0.02 *a*
Vaudray	32	17.4±0.4 *b*	58.3±3.4 *b*	1.51±0.03 *a*	1.08±0.01 *b*
High-contamination lakes					
Osisko	38	20.2±0.2 *b*	73.8±2.7 *b*	1.12±0.04 *bc*	0.88±0.01 *c*
Dufault	47	14.0±0.2 *c*	25.7±1.5 *c*	1.06±0.03 *c*	0.90±0.01 *c*

Note: Means followed by the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

Table 3. Concentrations of Zn, Cu, and Cd (mean ± SE, μg·g dry weight^{-1}) and correlations (Pearson's *r*) between concentrations of MT (nmol metal binding sites·g dry weight^{-1}) and metal (M) in pooled samples of liver or interrenal tissue of adult yellow perch collected in six lakes of the Rouyn-Noranda mining area.

	Liver				Interrenal tissue			
	N	[Zn]	[Cu]	[Cd]	*N*	[Zn]	[Cu]	[Cd]
Reference lakes								
Opasatica	8	92.4±3.6 *a*	10.4±1.8 *a*	2.9±0.4 *a*	8	104.6±2.3 *a*	2.3±0.2 *a*	0.9±0.1 *a*
Dasserat	7	98.6±4.2 *ab*	10.8±0.9 *a*	5.3±0.6 *b*	8	118.8±2.6 *b*	2.9±0.3 *a*	1.7±0.2 *b*
Intermediate-contamination lakes								
Bousquet	7	106.5±5.4 *ab*	20.4±4.5 *b*	20.3±2.9 *c*	8	96.08±1.2 *a*	2.2±0.1 *a*	3.8±0.2 *c*
Vaudray	8	108.9±1.6 *b*	12.9±0.7 *ab*	25.1±1.7 *c*	8	127.6±4.7 *b*	2.7±0.2 *a*	5.7±0.7 *cd*
High-contamination lakes								
Osisko	8	177.2±9.0 *c*	246.5±29.8 *c*	45.7±3.2 *d*	8	153.6±6.6 *c*	6.5±1.0 *b*	8.0±0.5 *de*
Dufault	8	151.1±3.7 *c*	148.5±11.1 *c*	61.3±5.3 *d*	4	227±10.3 *d*	6.4±1.5 *b*	12.6±0.4 *e*
Correlation between [MT] and [M]	46	0.93**	0.95**	0.91**	36	0.82**	0.77**	0.95**

Note: Means followed by the same letter are not significantly different; comparison between lakes only (Tukey–Kramer test, $P < 0.01$). **$P < 0.001$ (Pearson's test).

interrenal tissue also increased along the gradient of water and sediment contamination. The levels of Zn in the interrenal tissue were similar to concentrations in the liver, possibly reflecting basal homeostatically regulated physiological concentrations. However, Cu and Cd levels were much greater in the liver than in the interrenal tissue: in yellow perch from the most contaminated lakes, Cu levels in liver were 25- to 35-fold higher than the interrenal tissue levels. Studies in salmonids have shown higher Cd concentrations in the kidney compared with the liver after chronic exposure to Cd in the laboratory (Harrison and Klaverkamp 1989) and in the field (Farag et al. 1995). Preliminary analyses of kidney metal concentrations suggested that the tissue distribution was similar in wild yellow perch (H. Levesque, Département des Sciences Biologiques, Université du Québec à Montréal, personal communication). Thus, it could be expected that Cd levels in the interrenal tissue, which is located in the head kidney, would be greater than liver concentrations. However, our data clearly showed that Cd accumulates more in the liver of yellow perch than in the head kidney (around a fivefold difference in the most contaminated lakes).

The metal exposure of the yellow perch was further characterized by measures of MT. MT has been proposed in various studies as a biomarker of exposure to base metals. In Canada, the use of MT to monitor the effects of the mining industry is under technical evaluation (Couillard and St-Cyr 1997). Our data showed a significant difference in MT levels between reference, intermediate-contaminated, and highly contaminated lakes in the liver and in the interrenal tissue. Liver MT levels of yellow perch from the contaminated lakes were 10-fold higher than those of yellow perch from the reference lakes; interrenal MT levels were about threefold higher. This was the first study to measure MT levels in the interrenal tissue of wild fish and to demonstrate its increase related to metal levels. However, to prove unequivocally that MT is synthesized de novo in the interrenal tissue, it would be necessary to detect MT mRNA in the interrenal tissue and characterize the structure of the interrenal MT.

There is some evidence in the literature that MT levels correlate with metal (Zn, Cu, or Cd) levels in the liver of salmonids and European perch exposed in the laboratory or in the field (Hogstrand et al. 1991; Marr et al. 1995; Dallinger et al. 1997). In our study, MT levels in the liver and the interrenal tissue correlated strongly with the levels of Cd, Zn, or Cu. Since all three metals increased gradually in both tissues of yellow perch from the six lakes, no one metal could be identified as a putative inducer of MT synthesis. It is interesting that MT in the interrenal tissue strongly correlated in a linear way with Cu and Cd summation, but with the addition of Zn to the correlation, MT pro-

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Fig. 1. MT concentrations (mean ± SE) in pooled samples of (*a*) liver and (*b*) interrenal tissue of adult yellow perch collected from six lakes of the Rouyn-Noranda mining region. Means followed by the same letter are not significantly different (Tukey–Kramer test, $P < 0.01$). For each lake, $N = 8$ pooled samples. OP, Lake Opasatica; DS, Lake Dasserat; BO, Lake Bousquet; VA, Lake Vaudray; OS, Lake Osisko; DT, Lake Dufault. Lakes were ranked as reference (R), intermediate contaminated (I), or highly contaminated (H) according to the concentrations of metals accumulated in tissues.

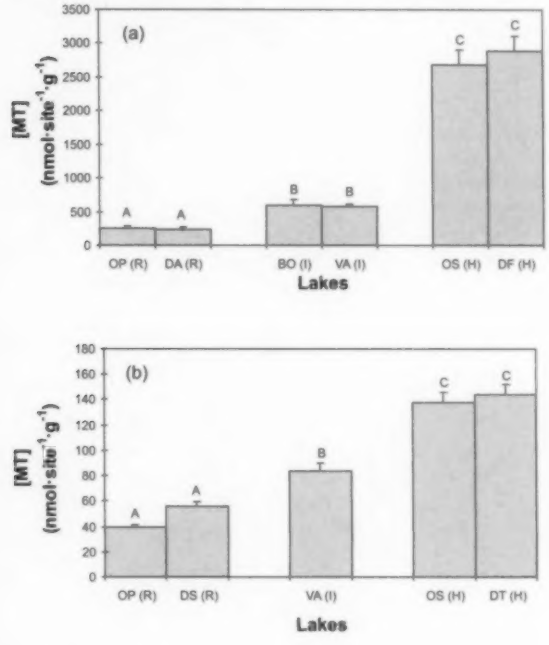
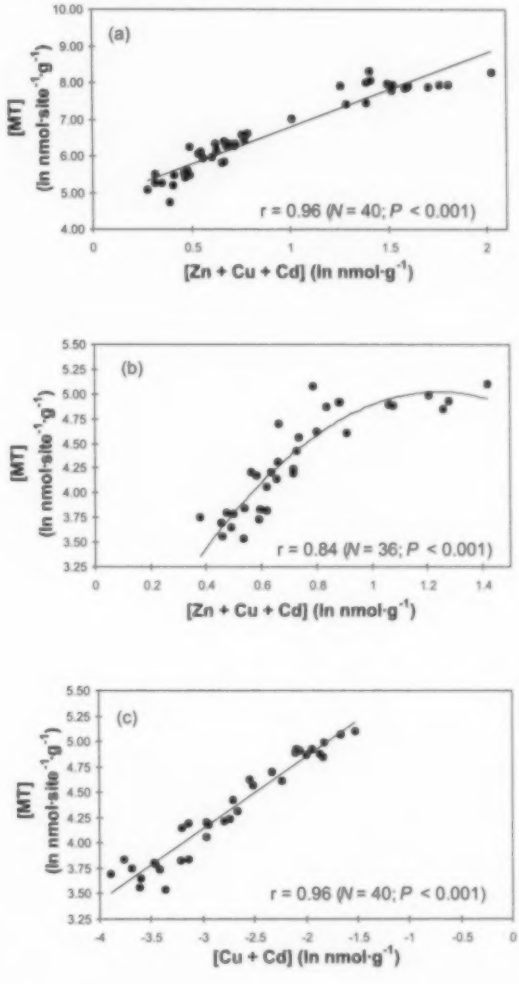


Fig. 2. Relationship (Pearson's *r*) between MT concentrations (ln nmol metal binding sites·g dry weight^{-1}) and summation of metals (ln nmol·g dry weight^{-1}) in combined samples of (*a*) liver and (*b* and *c*) interrenal tissue in adult yellow perch collected from six lakes of the Rouyn-Noranda mining region. OP, Lake Opasatica; DS, Lake Dasserat; BO, Lake Bousquet; VA, Lake Vaudray; OS, Lake Osisko; DT, Lake Dufault.


(a)
[MT] (ln nmol·site⁻¹·g⁻¹)
r = 0.96 (N = 40; P < 0.001)
[Zn + Cu + Cd] (ln nmol·g⁻¹)
(b)
[MT] (ln nmol·site⁻¹·g⁻¹)
r = 0.84 (N = 36; P < 0.001)
[Zn + Cu + Cd] (ln nmol·g⁻¹)
(c)
[MT] (ln nmol·site⁻¹·g⁻¹)
r = 0.96 (N = 40; P < 0.001)
[Cu + Cd] (ln nmol·g⁻¹)

duction reached a plateau. This might indicate a Zn spillover onto other cytosolic ligands, but further investigations of cytotoxicity and subcellular partitioning of the metals would be needed to support this hypothesis.

Once the metal exposure and the MT response had been quantified in the liver, an important site of metal accumulation in the fish (Heath 1995), and in the interrenal tissue, a component of the hypothalamo–pituitary–interrenal axis, the link between contamination and physiological effects could be investigated. The physiological capacity to respond to a stressor is a fundamental endocrine response (Hontela 1997; Wendelaar Bonga 1997), and the results of our in vivo and in vitro functional tests of the interrenal tissue indicated an impairment of the cortisol stress response in adult yellow perch from the highly contaminated lakes. Following the standardized confinement stress test, yellow perch from the two most contaminated lakes had lower plasma cortisol levels than those from the reference lakes. Three previous studies in metal-contaminated sites also showed lower cortisol response either postcapture (Lockhart et al. 1972; Brodeur et al. 1997*a*) or after a 1-h confinement (Norris et al. 1999). If we compare the pattern of plasma cortisol levels in confinement-stressed yellow perch and MT levels, in relation to contamination, MT appears to be more sensitive as an indicator of metal exposure, since a significant difference is detected between each degree of contamination, while a difference in the capacity to generate the cortisol stress response is observed only between reference and the most contaminated lakes. This finding conforms to previous models of sensitivity at different levels of biological organization (Adams 1990), since the endocrine stress response represents a higher level of response than induction of MT, a biomarker of exposure. The in vivo evidence for an interrenal dysfunction in yellow perch chronically exposed to high ambient metal concentrations in the field was further supported by the in vitro experiment. Cortisol secretion of the interrenal tissue stimulated by optimal concentrations of ACTH or dbcAMP, an agonist of the second messenger cAMP, was

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Table 4. Plasma chloride and glucose (means ± SE (*N*)) in an unstressed group and a stressed group (subjected to a standardized stress test) of adult yellow perch collected from three lakes of the Rouyn-Noranda mining area.

	Chloride ($mg \cdot mL^{-1}$)		Glucose ($mg \cdot mL^{-1}$)	
Lake	No stress	Stress	No stress	Stress
Opasatica (reference)	141±4 (22)	126±4 (29)*	0.14±0.02 (22)	0.26±0.02 (31)*
Vaudray (intermediate)	146±16 (11)	129±8 (29)	0.33±0.03 (11)*	0.44±0.02 (31)*#
Osisko (high)	149±6 (21)	116±2 (31)*	0.18±0.03 (21)	0.18±0.01 (38)#

Note: Lakes were ranked as reference, intermediate contaminated, or highly contaminated according to the concentrations of metals accumulated in tissues. *Significantly different from the no-stress group (*t* test, $P < 0.05$); #significantly different from the other two lakes (*t* test, $P < 0.05$).

Fig. 3. Plasma cortisol levels (mean ± SE) after a standardized stress test in adult yellow perch collected from six lakes of the Rouyn-Noranda mining region. Means followed by the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$). Numbers of fish sampled in each lake are indicated inside the bars. Lakes were ranked as reference (R), intermediate contaminated (I), or highly contaminated (H) according to the concentrations of metals accumulated in tissues.

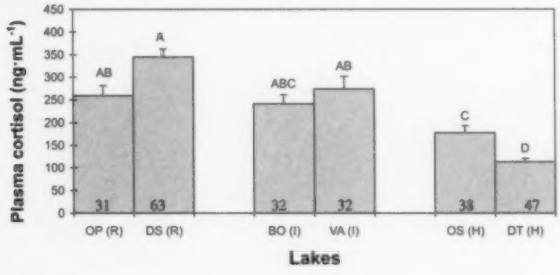
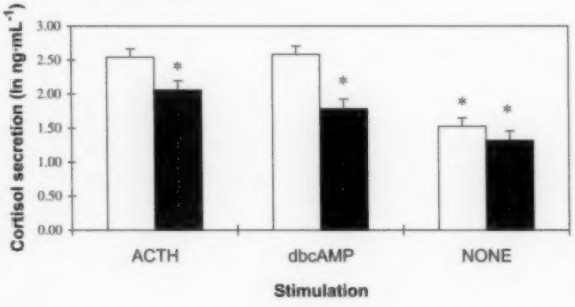


Fig. 4. In vitro cortisol secretion (least squares mean ± SE for the mean value of basal cortisol secretion before stimulation) of the interrenal tissue of adult yellow perch from a reference lake (Lake Opasatica, open bars, $N = 22$) and a contaminated lake (Lake Osisko, solid bars, $N = 17$) stimulated by 2 IU of porcine ACTH, 4 mmol of dbcAMP, or not stimulated. *Significantly different from the stimulated interrenal tissue of the reference lake (ANCOVA test, $P < 0.05$).



also lower in yellow perch from the most contaminated lake. It is the capacity to respond to stimulation that was different between interrenals from the reference and contaminated lakes; the basal cortisol secretion was the same. The interest in comparing cortisol output following ACTH, which interacts with a membrane receptor, or dbcAMP, which diffuses directly into the cells, bypassing the membrane receptor, was to determine if cortisol secretion in fish exposed to metals is affected at the membrane receptor or postmembrane steps. Indeed, since cortisol secretion in response to ACTH was impaired in interrenals from fish sampled in Lake Osisko, the most contaminated lake, and stimulation with dbcAMP could not restore the secretory response, we can postulate that metals disrupt cortisol biosynthesis at multiple steps, including postmembrane steps. Similar results were obtained in a laboratory study using rainbow trout, *Oncorhynchus mykiss*, interrenal cell suspensions exposed in vitro to Cd and Zn (Leblond and Hontela 1999).

Even though further studies are needed to elucidate the precise mechanisms through which chronic exposure to metals alters the normal capacity to generate the hormonal stress response, the present study provided new data linking field exposure to metals to an endocrine dysfunction. The physiological consequences of this dysfunction were also investigated in the present study. Lower plasma chloride levels have been reported in wild fish from metal-contaminated sites or in fish exposed to metals in laboratory experiment (Larsson et al. 1985). Cortisol promotes proliferation and differentiation of chloride cells in the gills, increasing chloride body influxes but not plasma chloride levels (Laurent and Perry 1990; Madsen 1990). Despite differences in cortisol status and in tissue metal burdens between fish from different lakes in our study, difference in chloride levels were not detected. Stressed fish did, however, have lower plasma chloride levels compared with unstressed fish from the same lake, probably reflecting osmoregulatory disturbances due to the 1-h confinement stress.

A rise in plasma glucose is another expected response to stress (Barton and Iwama 1991). In our study, stressed fish from the reference and intermediate lakes were able to raise their plasma glucose levels compared with unstressed fish from the same lake, but this capacity was significantly inhibited in yellow perch from the most contaminated lake. The mechanisms underlying this metabolic dysfunction are under investigation in our laboratory; disruption of corticosteroid or catecholamine synthesis, abnormal gluconeogenesis, and (or) exhaustion of the energy reserves are considered. Fish from the two most contaminated lakes also had a lower condition factor, an indication of decreased energy reserves (Goede and Barton 1990). Many biotic and abiotic variables, other than xenobiotics, influence the growth and condition factor of fish. However, there is some evidence suggesting a significant metabolic cost associated with resisting chemical contaminants (Heath 1995). A recent study by Sherwood et

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al. (2000) provided evidence that yellow perch from the two most contaminated lakes (Lakes Osisko and Dufault) have lower growth efficiency (capacity to convert consumed food into body mass) compared with yellow perch from the reference lakes. Our data on lower condition of the same yellow perch populations gave further support to the evidence for growth impairment in yellow perch subjected to chronic environmental exposures to metals.

This was the first study to characterize metals and MT levels in the interrenal tissue of wild fish and to demonstrate that metals increase in the interrenal tissue in relation to exposure, that MT is present in this tissue, and that its levels are related to tissue metal concentrations. Clear differences in MT levels between reference, intermediate-contaminated, and highly contaminated lakes were observed in the liver and the interrenal tissues of yellow perch. There was also an exposure-related impairment of the cortisol response in vivo and of the capacity to respond to ACTH and dbcAMP in vitro. In addition, fish from the most contaminated lakes exhibited a metabolic impairment characterized by a lower capacity to increase plasma glucose in response to a confinement stress and a lower condition factor.

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Population genetics, phylogeography, and systematics of the thornyhead rockfishes (*Sebastolobus*) along the deep continental slopes of the North Pacific Ocean

Carol A. Stepien, Alison K. Dillon, and Amy K. Patterson

Abstract: Population genetic, phylogeographic, and systematic relationships are elucidated among the three species comprising the thornyhead rockfish genus *Sebastolobus* (Teleostei: Scorpaenidae). Genetic variation among sampling sites representing their extensive ranges along the deep continental slopes of the northern Pacific Ocean is compared using sequence data from the left domain of the mtDNA control region. Comparisons are made among the shortspine thornyhead (*S. alascanus*) (from seven locations), the longspine thornyhead (*S. altivelis*) (from five sites), which are sympatric in the northeast, and the broadbanded thornyhead (*S. macrochir*) (a single site) from the northwest. Phylogenetic trees rooted to *Sebastes* show that *S. macrochir* is the sister taxon of *S. alascanus* and *S. altivelis*. Intraspecific genetic variability is appreciable, with most individuals having unique haplotypes. Gene flow is substantial among some locations and others diverged significantly. Genetic divergences among sampling sites for *S. alascanus* indicate an isolation by geographic distance pattern. Genetic divergences for *S. altivelis* are unrelated to the hypothesis of isolation by geographic distance and appear to be more consistent with the hypothesis of larval retention in currents and gyres. Differences in geographic genetic patterns between the species are attributed to life history differences in their relative mobilities as juveniles and adults.

Résumé : Nous nous sommes penchés sur les relations démogénétiques, phylogéographiques et systématiques entre les trois espèces composant le genre des sébastolobes (*Sebastolobus* : Teleostei : Scorpaenidae). Nous comparons la variation génétique entre des stations d'échantillonnage représentant les vastes aires de répartition de ces espèces le long des pentes continentales profondes du Pacifique Nord à l'aide de données de séquençage du domaine gauche de la région régissant l'ADN mitochondrial. Les comparaisons portent sur le sébastolobe à courtes épines *S. alascanus* (sept stations), le sébastolobe à longues épines *S. altivelis* (cinq stations), qui sont sympatriques dans le nord-est, et le sébastolobe à larges bandes *S. macrochir* (une seule station) du nord-ouest. Les arbres phylogénétiques rattachés à *Sebastes* montrent que *S. macrochir* est un taxon frère de *S. alascanus* et *S. altivelis*. La variabilité génétique intraspécifique est notable, la plupart des individus présentant des haplotypes particuliers. Le flux génique est important entre certaines stations, alors que d'autres divergent de façon significative. Les divergences génétiques entre les stations d'échantillonnage pour *S. alascanus* indiquent un isolement par écartement géographique. Pour *S. altivelis*, elles n'ont pas de lien avec l'isolement géographique, et semblent plutôt correspondre à l'hypothèse de la rétention des larves par les courants et les gyres. Les différences dans les patrons génétiques au plan géographique entre les espèces sont attribuables à des différences d'ordre biologique dans la mobilité relative des juvéniles et des adultes.

[Traduit par la Rédaction]

Introduction

The thornyhead rockfishes, genus *Sebastolobus* (Teleostei: Scorpaenidae), have extensive ranges along the deep continental slopes of the northern Pacific Ocean (Eschmeyer et al. 1983; Matsuda et al. 1984). The thornyheads are abundant and support a large commercial ground fishery (Matsuda et al. 1984; Gunderson 1997). *Sebastolobus* comprises three species and is hypothesized to be a derived genus descended from the species-rich genus *Sebastes* (Washington et al. 1984).

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C.A. Stepien,[1] A.K. Dillon,[2] and A.K. Patterson.[3] Department of Biology, Case Western Reserve University, Cleveland, OH 44106-7080, U.S.A.

[1]Author to whom all correspondence should be addressed. Present address: Center for Environmental Science, Technology and Policy, Cleveland State University, 1899 East 22nd Street, MC 219, Cleveland, OH 44114-4435, U.S.A.
e-mail: c.stepien@csuohio.edu
[2]Present address: Athersys, Inc., 3201 Carnegie Ave., Suite 280, Cleveland, OH 44115, U.S.A.
[3]Present address: Department of Biokinesiology and Physical Therapy, University of Southern California, Los Angeles, CA 90089, U.S.A.

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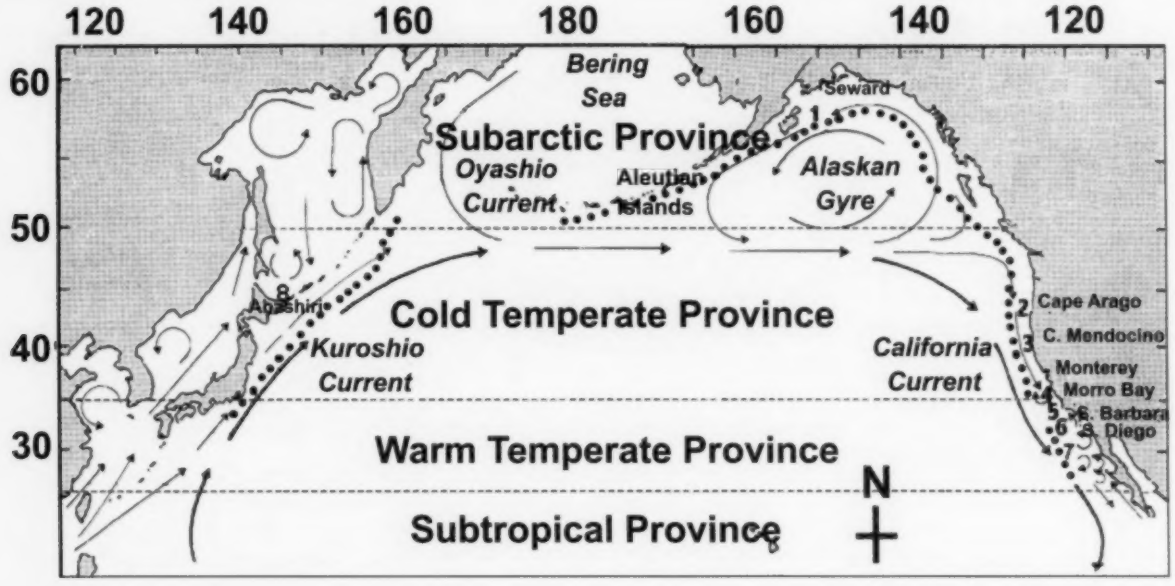
Fig. 1. Map showing sample sites (numbers) and species ranges (dots) for *Sebastolobus alascanus*, *S. altivelis*, and *S. macrochir* in the North Pacific Ocean. Biogeographic provinces (adapted from Briggs 1974) and major current patterns in the collection regions (adapted from Huyer et al. 1989) are indicated.



Adult shortspine thornyhead (*S. alascanus*) and longspine thornyhead (*S. altivelis*) inhabit depths from 600 to 1000 m and are sympatric from the Aleutian Islands, Alaska, to central Baja California, Mexico, although the latter species is rare in the north (Fig. 1) (Eschmeyer et al. 1983). The broadbanded thornyhead (*S. macrochir*) ranges from Sagami Bay to the southern Kuril Islands and Sakhalin, Japan, occupying depths from 150 to 500 m (Fig. 1) (Matsuda et al. 1984). The extensive latitudinal ranges of thornyheads permitted the present study to test whether population genetic relationships of deep-sea fishes are influenced by major oceanic circulation patterns near the surface (such as the Alaskan Gyre and the California Current) (Fig. 1) (Huyer et al. 1989). Their genotypes may thus be distributed widely during their pelagic early life history stages (Sinclair 1988), and relationships among geographic areas may reflect an isolation by geographic distance model.

Thornyheads have long generation times and longevities (over 100 years in *S. alascanus* and over 40 years in *S. altivelis*; Butler et al. 1995). Their eggs are fertilized in situ and float to surface waters (Moser 1974). Their extended pelagic larval stages, ranging from 14 to 15 months for *S. alascanus* and from 18 to 20 months for *S. altivelis* (Moser 1974; Wakefield 1990), may result in high levels of gene flow and diminishing genetic structure among populations. Their early life history stages are expected to be widely transported, largely by the Alaskan Gyre system in the north and by the California Current moving from north to south (Fig. 1). The currents and gyres may also retain eggs, larvae, and juveniles (Sinclair 1988), resulting in genetic divergences among those systems. Butler et al. (1996) postulated that the late larval and juvenile stages, which are found in midwater, are transported northward in the California Countercurrent, compensating for the initial southward transport. These movements may result in the retention of larvae and later recruitment nearer to adult populations, counteracting gene flow.

The member/vagrant hypothesis (Sinclair 1988) states that marine larvae that settle in appropriate habitats are passively and (or) actively retained in coastal current patterns (see Fig. 1), resulting in genetic divergence among adult populations from different regions. Members are individuals that are the most fit and recruit to appropriate habitats housing other adults, while vagrants have greater dispersal and risk being lost from the gene pool. Barriers to dispersal by major currents and the corresponding larval retention driving successful recruitment would produce geographic clustering and regional divergence of genotypes. Hence, gene flow and genetic divergence in marine animals with planktonic larvae may be structured by these larval retention areas, which would be reflected in the genetic divergences among adult populations. If larval retention occurs, then genetic relationships among populations may be related to current and gyre patterns (Fig. 1) and may show inconsistencies with an isolation by geographic distance model. Larval retention in some areas would result in significant geographic differences among some populations, if it occurred in some areas but not in others. These patterns would be related to selection, in that larvae that were swept away from appropriate adult habitats would be lost to the gene pool but would be reflected in selectively "neutral" gene regions such as the mtDNA control region examined here (Stepien and Kocher 1997). If retention occurs in given areas over many generations and gene flow among areas is reduced, then closely spaced geographic areas will reveal clusters of genetically related genotypes and (or) significantly different frequencies of given genotypes. The retention areas thus would serve as vicariant barriers to gene flow, reducing panmixia.

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Juvenile *S. altivelis* settle on the slope at depths between 600 and 1200 m and apparently do not migrate as juveniles or adults (Wakefield 1990). In contrast, juvenile *S. alascanus* settle on the substrata at about 100 m and then move to deeper waters along the slope with age (Moser 1974; Wakefield 1990). The ontogenetic migration of *S. alascanus* down the slope may result in increased gene flow as compared with *S. altivelis*.

A companion study, also using mtDNA control region sequences, examined genetic variation of the sympatric continental slope Dover sole (*Microstomus pacificus*) from the northeastern Pacific and its congener the slime sole (*Microstomus achne*) from the northwest (Stepien 1995, 1999). Significant geographic partitioning among several sampling sites for *M. pacificus* suggested larval retention and was inconsistent with the hypothesis of isolation by geographic distance (Stepien 1999). The present investigation tests whether similar biogeographic patterns of population genetic variation occur in the thornyheads.

Thornyheads are harvested by a deepwater bottom trawl fishery that also targets Dover sole (Gunderson 1997). The slope benthos is heavily scoured by trawl marks. Due to their slow growth rates and extensive ages (Butler et al. 1995), slope fishes may be highly vulnerable to overexploitation (Stepien 1995). Little is presently known about the ecology, life histories, and capacity of thornyhead populations to withstand fishing pressure (Jacobson and Vetter 1996). An understanding of their genetic population structure may be important to maintain the fishery and to determine which areas of the continental slope habitat are particularly important to their reproduction.

The objectives of the present study are to compare the population genetic structures and phylogeographic and systematic relationships among the three species of *Sebastolobus* by using sequences of the left domain of the mtDNA control region. Systematic relationships of the thornyheads have not been previously investigated.

Little is known about the population genetic structure of continental slope fishes (Siebenaller 1978; Stepien 1995; Smith et al. 1997). Our primary null hypothesis is that there are no significant geographic patterns of genetic divergence in thornyheads along the continental slope (thus, panmixia is supported). The alternative hypothesis is that patterning occurs, suggesting either larval retention in currents and gyres or isolation by geographic distance that is linked to major oceanic circulation patterns. A secondary null hypothesis is that the shortspine and longspine thornyheads show similar patterning. The alternative to this secondary hypothesis is that the relationships among areas are different for different species, possibly due to differences in dispersal or to life history variations.

Materials and methods

Sampling sites

Sampling locations in the northeastern Pacific encompass three biogeographic provinces (Fig. 1) (Briggs 1974) based on surface water temperatures: the subarctic (represented by a location in the Bering Sea at Seward, Alaska), the cold temperate (including Cape Arago in Oregon, Cape Mendocino in northern California, and Monterey Bay and Morro Bay in central California), and the warm temperate zones (from the Santa Barbara channel and San Diego in southern California; see Fig. 1). A total of 93 shortspine thornyheads from seven sites representing most of their range were sampled and sequenced, including fish from Seward (N = 14), Cape Arago (N = 13), Cape Mendocino (N = 12), Monterey Bay (N = 14), Morro Bay (N = 13), Santa Barbara (N = 10), and San Diego (N = 17). A total of 55 longspine thornyheads were sampled and sequenced from five of the same sites, including Cape Arago (N = 6; they are less common in the north), Cape Mendocino (N = 16), Morro Bay (N = 12), Santa Barbara (N = 9), and San Diego (N = 12). Fourteen broadbanded thornyheads were sampled and sequenced from a site off Abashiri, Japan (Fig. 1). Sequences of *Sebastolobus* were compared with those published for a scorpaenid outgroup, *Sebastes marinus* (Lee et al. 1995).

DNA extraction, amplification, and sequencing

Liver and muscle tissues were either fresh frozen in liquid nitrogen or frozen on dry ice and stored at –80°C. Frozen tissues were ground in liquid nitrogen using a cylindrical stainless steel mortar and pestle. DNA was extracted and purified following Stepien (1995). The left domain of the mtDNA control region was amplified by the polymerase chain reaction using methodology previously described (Stepien 1995, 1999). Both DNA strands were sequenced separately for accuracy.

Data analysis

Variable characters among taxa included base substitutions (transitions and transversions) and insertions and deletions (indels). Levels of inter- and intra-population genetic diversity were quantified by haplotype diversity (h) (Nei and Tajima 1983) and the average number of nucleotide substitutions per nucleotide site within (nucleotide diversity (π) (Nei 1987) and among populations (nucleotide divergence (d_{xy}) (Nei and Tajima 1983), based on the entire data set using the program DA2 in restriction enzyme analysis package (REAP) version 5.0 (McElroy et al. 1992; McElroy 1997). Differences in nucleotide substitution frequencies among species were tested with chi-square contingency table tests (Sokal and Rohlf 1995).

Analysis of molecular variance (AMOVA) version 1.55 (Excoffier 1995) tested for a hierarchical partitioning of genetic variability among versus within sampling sites and biogeographic provinces. AMOVAs utilized Euclidean distances based on the numbers of nucleotide differences among pairs of the haplotypes. AMOVA computed F statistic analogs (ϕ_{ST}) and used 1000 randomly permutated matrices to test their significance (Excoffier et al. 1992). Sampling sites and (or) biogeographic provinces were considered significantly different from one another if the measured variance was lower than 95% of the variance in the null distribution (Excoffier et al. 1992). A Bonferroni correction was employed for multiple pairwise comparisons among locations (Sokal and Rohlf 1995). AMOVAs were done on the entire haplotype data sets for *S. alascanus* and *S. altivelis*. Since most individuals had unique haplotypes, the number of haplotypes in the data set was reduced by using only transversional substitutions, resulting in a greater number of shared haplotypes (following Bowen and Grant 1997; Stepien 1999). AMOVAs were repeated using the transversion haplotypes. The relationship of ϕ_{ST} (from the AMOVAs) and geographical distances (kilometres) between pairs of sample sites based on the transversion haplotypes was tested with correlation and least-squares regression analyses (Sokal and Rohlf 1995) using Microsoft Excel 97.

The evolutionary relationships among haplotypes and their divergences were measured by determining uncorrected pairwise distances and their standard errors and clustering with the neighbor-joining (NJ) algorithm in phylogenetic analysis using parsimony (PAUP)* 4.0 version d64 (Swofford 1998). NJ analyses were done separately based on the entire data set and then restricted to transversions and indels (the latter following Bowen and Grant

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Fig. 2. MP trees of relationships among thornyhead taxa and percentages show results of 500 bootstrap replications from PAUP* 4.0 version d64 (Swofford 1998) that are above 50%. The NJ trees based on pairwise distances had identical topologies and are not shown. Vertical lines denote synapomorphies. (*a*) Single most parsimonious tree of relationships among the three species of *Sebastolobus* based on the overall data set (excluding characters that were polymorphic within a species) using an exhaustive search. Length = 139 steps, CI excluding uninformative characters = 0.84, and $g1$ skewness = –0.71. This topology was obtained with all weighting regimes. (*b*) Majority-rule consensus of 410 most parsimonious trees from a branch and bound search among *S. alascanus* haplotypes, with *S. altivelis* and *S. macrochir* used as designated outgroups. Consensus values are given above the nodes. Synapomorphies that distinguish groups of taxa are denoted by vertical lines at the nodes, and their nucleotide numbers and base composition are indicated below the nodes. Lengths = 220 steps, CI excluding uninformative characters = 0.32, and 43 variable characters were parsimony informative. The results were identical with all weighting regimes. Based on transversions only and indels with a branch and bound search (tree not shown), there were 12 most parsimonious trees with 89 steps, CI excluding uninformative characters = 0.52, and 29 characters that were parsimony informative. Vertical lines next to taxa designate groups of geographically related haplotypes. (*c*) Majority-rule consensus of 300 most parsimonious trees based on a branch and bound search among *S. altivelis* haplotypes, with *S. alascanus* and *S. macrochir* used as designated outgroups. Consensus values are given above the nodes. Synapomorphies that distinguish groups of taxa are denoted by vertical lines at the nodes, and their nucleotide numbers and base composition are indicated below the nodes. Lengths = 105 steps, CI excluding uninformative characters = 0.52, and 37 variable characters were parsimony informative. The results were identical with all weighting regimes. Letters designate individual haplotypes based on the entire data set (nucleotide substitutions are indicated in the Appendix), and numbers indicate transversion haplotypes (see Table 1). Based on transversions and indels only with an exhaustive search (tree not shown), there were six most parsimonious trees with 60 steps, CI excluding uninformative characters = 0.70, and 17 variable characters that were parsimony informative. Vertical lines next to taxa designate groups of geographically related haplotypes.

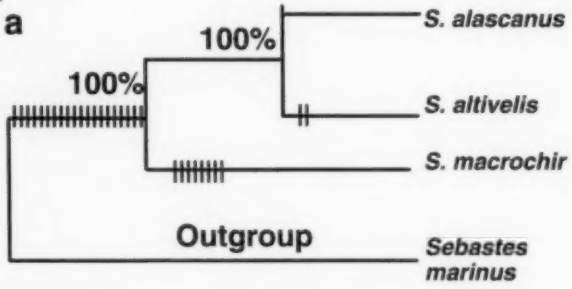
1997). Character state relationships and their evolutionary patterns also were analyzed with maximum parsimony (MP) in PAUP*. Phylogenetic analysis among the three species was based on an exhaustive search using the entire data set (with deletion of characters that were polymorphic within a species). *Sebastes marinus* (from Lee et al. 1995) was designated as the outgroup. Three separate runs were done for all analyses, including equal weighting of all types of characters (transitions, transversions, and indels), with indels and transversions weighted five times that of transitions, and including only indels and transversions. Relationships among the haplotypes for *S. alascanus* and *S. altivelis* were determined from separate branch and bound searches, and the most common haplotypes of the other species and *S. macrochir* were designated as outgroups. Relationships supported by multiple most parsimonious trees were presented by a 50% majority rule consensus tree (Swofford et al. 1996). Bootstrap analyses were used to compute support for individual nodes of the NJ (with 1000 replications) and MP trees (with 500 replications in a heuristic search).

A molecular clock calibration of 2% pairwise sequence divergence per million years (my) was used for the left domain of the mtDNA control region in order to compare the ages of lineages. This rate is slower than that of the mtDNA control region in mammals (reviewed by Avise 1994) but is average for fishes, whose mtDNA evolves more slowly (Stepien and Kocher 1997).

Results

Characters and relationships among the species of *Sebastolobus*

The pairwise sequence divergence between *Sebastolobus* and *Sebastes* is $p = 0.25 \pm 0.01$, corresponding to a separation of about 12.5 my (using a rate calibration of 2%/my). Consensus sequences for the mtDNA control region for the three species of *Sebastolobus* and bases having intraspecific nucleotide substitutions and insertions/deletions among taxa are given in the Appendix. Consensus sequences are deposited in GenBank with accession numbers AF161803 for *S. alascanus*, AF161804 for *S. altivelis*, and AF161805 for *S. macrochir*. The species of *Sebastolobus* are united by 65 synapomorphies (not shared with the outgroup *S. marinus*), including 62 nucleotide substitutions and three insertions (at



nucleotide positions 249–251, 376, and 417; Appendix). Sequences of *S. marinus* (Lee et al. 1995) had insertions at positions 074–081 and 088–091 that are absent in *Sebastolobus*. The putative termination associated sequence (Doda et al. 1981) extends from nucleotide position 115 to position 130 and was invariable among the taxa, including *S. marinus*. The central conserved section extends from nucleotide 392 to nucleotide 461 and includes the conserved sequence block D from base 442 to base 463 (Appendix) (Doda et al. 1981).

Two separate nucleotide insertions in *S. altivelis* (at nucleotide positions 400 and 407 in *S. altivelis*; Appendix) distinguish the species from *S. alascanus*. A deletion is shared by *S. macrochir* and *S. altivelis* at nucleotide position 433, at which position an adenine occurs in *S. marinus* and *S. alascanus*. There are significant differences in the frequencies of five nucleotide polymorphisms for the total number of individuals of *S. alascanus* and *S. altivelis*. *Sebastolobus macrochir* is distinguished from *S. alascanus* and *S. altivelis* by 12 fixed differences, including five transversions, five transitions, and two insertions. Three of these characters are shared with *S. marinus* (Appendix).

NJ and MP trees based on the entire data set (Fig. 2*a*) resolve *S. macrochir* as the sister group to a clade comprising *S. alascanus* and *S. altivelis*. The lineage in the northeastern Pacific leading to *S. alascanus* and *S. altivelis* appears to have diverged from an ancestor shared with *S. macrochir* by a mean pairwise distance of $p = 0.090 \pm 0.020$, estimated as

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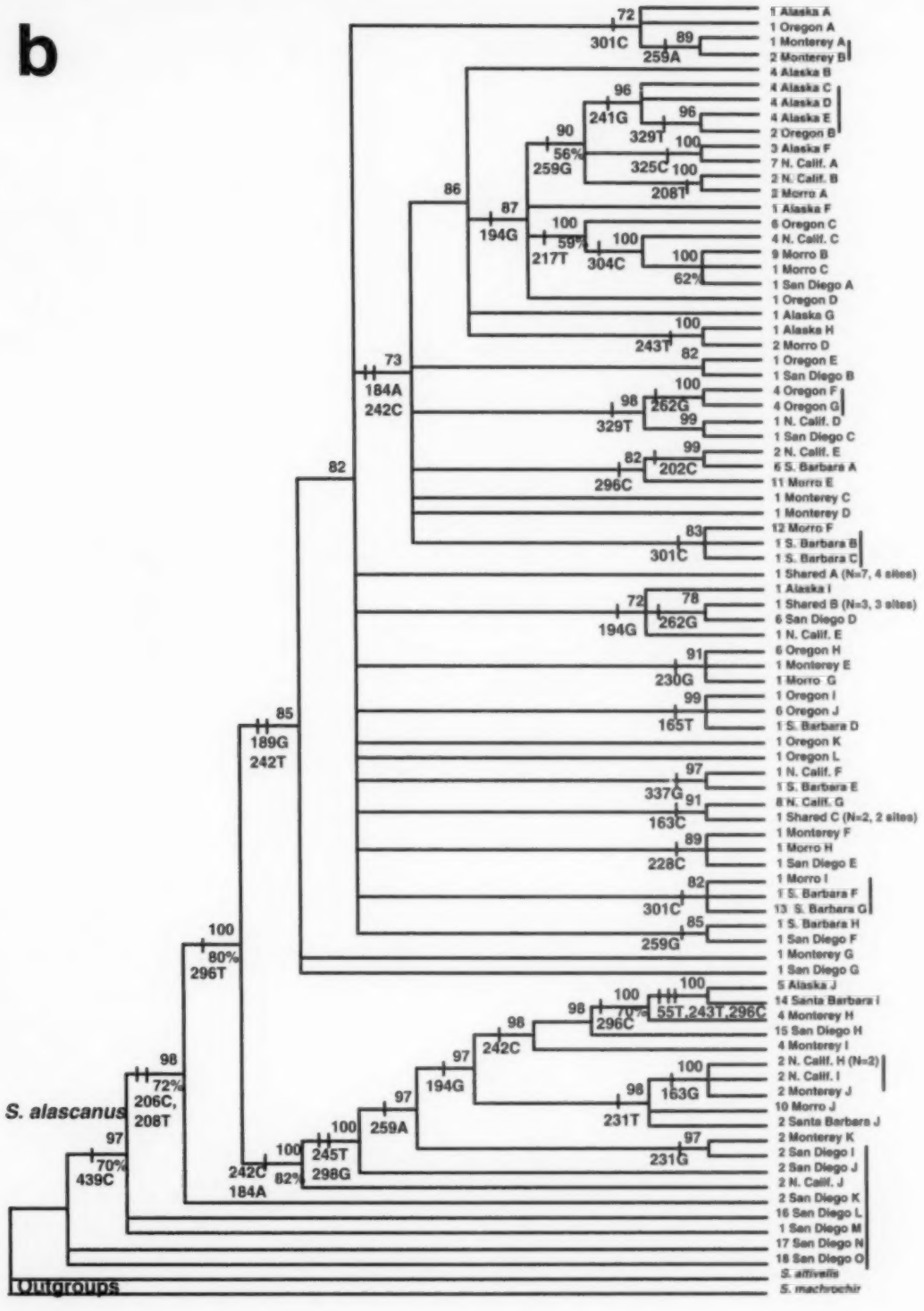

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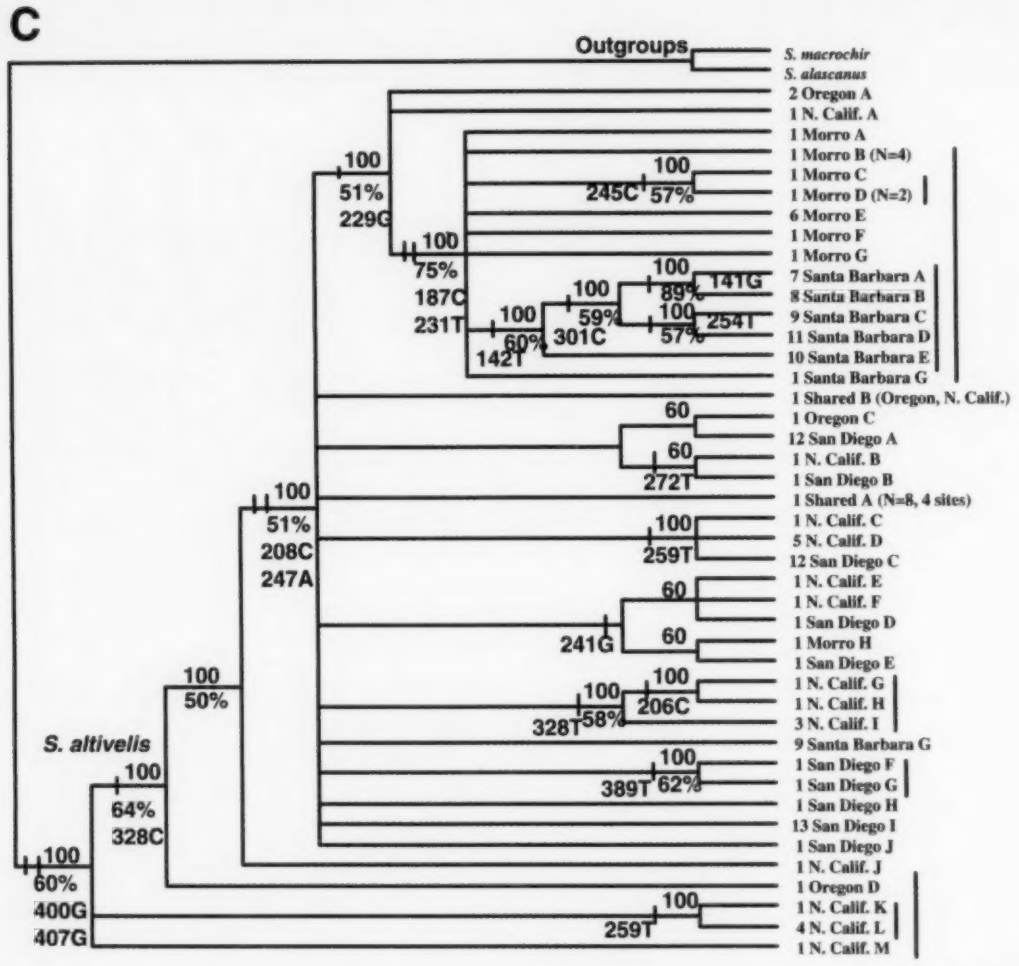

4–5 million years ago (mya). *Sebastolobus alascanus* and *S. altivelis* diverge by a mean pairwise distance of p = 0.026 ± 0.14, or about 1.3 my.

Genetic variability within and among populations

Based on the entire data set for the left domain of the mtDNA control region, four haplotypes are shared and 79 individuals are unique among 93 individual *S. alascanus*. Four haplotypes are shared among a total of 43 haplotypes from 55 individual *S. altivelis*. All of the 14 *S. macrochir* from Japan have unique haplotypes. Polymorphic nucleotides are indicated in the Appendix.

For *S. alascanus*, within-site haplotype diversity (h) from the entire data set averages 0.990 ± 0.001, ranging from 0.97 in Alaska to 1.0 in the Oregon, Monterey Bay, Santa Barbara, and San Diego sampling locations. Within-locality nucleotide diversity (π) averages 0.015 ± 0.006, ranging from 0.012 in Alaska to 0.018 in San Diego. Between pairs of sampling sites, π averages 0.015 ± 0.001 for *S. alascanus*, ranging from 0.010 to 0.018. Levels of nucleotide diversity within and between population sites are thus about equal. Mean nucleotide divergence (d_{xy}) among locations is 0.0004 ± 0.0001, ranging from 0.0001 between the Santa Barbara and Monterey Bay sites to a maximum of 0.0009 between the northern California and San Diego sites (Fig. 1).

In *S. altivelis*, h averages 0.960 ± 0.001 (ranging from 0.890 in Morro Bay to 1.000 in the Santa Barbara site). Within-site π is somewhat less in *S. altivelis* than in *S. alascanus*, averaging 0.009 ± 0.004 and ranging from 0.005 in Oregon to 0.014 in Santa Barbara. Between-site π averages 0.012 ± 0.009 for *S. altivelis*, ranging from 0.006 to 0.016. Average d_{xy} among locations is 0.003 ± 0.004, ranging from 0.0001 between the Oregon and San Diego locations to 0.0058 between Morro Bay and San Diego (Fig. 1). In *S. macrochir*, h is 1.0, since all haplotypes are unique in the single site surveyed. Within-site π is 0.016.

The mean sequence divergences among pairs of haplotypes within the species are p = 0.013 ± 0.008 (about 650 000 years) for *S. alascanus* and p = 0.014 ± 0.008 (about 700 000 years) for *S. altivelis*. Intraspecific divergences among haplotypes of *S. macrochir* average p = 0.015 ± 0.008, about 750 000 years for the single site surveyed.

Relationships and divergences among populations

The NJ and MP trees from the entire data set and those for haplotypes based on transversions and indels (indels oc-

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curred only among the different species; Table 1) resolve few haplotype groupings for *S. alascanus* that correspond to sampling sites or to biogeographic areas. MP analyses of the entire data set based on repeated heuristic searches yield 410 most parsimonious trees, for which the 50% majority rule consensus tree is given in Fig. 2*b*. Haplotypic relationships of *S. alascanus* do not reveal a discernable geographic pattern (Fig. 2*b*) in any of the analyses. In contrast, five haplotypes of *S. altivelis* from Santa Barbara (representing six of nine individuals sampled from that location) group together in a clade united by a common transversion at nucleotide position 142 (Table 1*b*). This *S. altivelis* clade is resolved both with the entire data set (Fig. 2*c*) and with transversion and indel haplotypes (Table 1). Those haplotypes then form a larger clade with haplotypes from the neighboring site of Morro Bay in the NJ and MP trees using the entire data set, characterized by two transitional synapomorphies at nucleotide positions 187 and 231 (Fig. 2*c*).

AMOVAs reveal significant differences in the frequencies of transversion haplotypes (given in Table 1) among some pairs of sampling sites for *S. alascanus* and *S. altivelis* (Table 2). Based on the entire haplotype data sets, results are not significant due to the large numbers of haplotypes and the lack of shared haplotypes. For *S. alascanus*, significant differences based on the transversion haplotypes include comparisons between the site in northern California versus those in Oregon, Morro Bay, Santa Barbara, and San Diego, but no difference is found from the next most southerly location in Monterey Bay (Table 2). The sample from Alaska differs significantly from samples at Morro Bay and Santa Barbara but not from samples from the more northerly sites or from the most southerly location (San Diego). In *S. altivelis*, significant differences occur between the samples from Santa Barbara versus all other sites, based on transversion haplotypes (Table 2).

AMOVAs of frequencies for the transversion haplotypes show that 94.3% of the genetic variance in *S. alascanus* occurs within locations and that there is significant partitioning of variation among sample sites (4.4%) and less among putative biogeographic provinces (1.3%). For *S. altivelis*, 72.2% of the genetic variance occurs within locations. Variance also is significantly partitioned among sites (24.0%) and less among the biogeographic provinces (3.8%; not significant but greater than for *S. alascanus*). Overall, results show significant divergences in the distribution of haplotypes among sampling locations for both species, indicating moderate levels of genetic divergence (Nei 1987) and different patterns in each species (Table 2).

Correlation and least-squares regression analyses (Sokal and Rohlf 1995) using Microsoft Excel 97 show significant a relationship between ϕ_{ST} and geographical distances for *S. alascanus* (Fig. 3*a*) but not for *S. altivelis* (Fig. 3*b*).

Discussion

Evolutionary and phylogeographic relationships of *Sebastolobus*

Fossil *Sebastes* from the Lompoc deposits on the central California coast date to the middle Miocene epoch (Barsukov 1991), corresponding to our divergence estimate between *Sebastes* and *Sebastolobus* of about 12.5 my. By comparison, the nucleotide divergence between *Sebastes* and *S. alascanus* measured from mtDNA cytochrome *b* gene sequences was $p = 0.16$ (Johns and Avise 1998), indicating that cytochrome *b* (a coding region) evolves more slowly than the control region (at about 1.56%/my), as appears to be true in other teleosts that have been studied (reviewed by Stepien and Kocher 1997).

The northeastern Pacific lineage including *S. alascanus* and *S. altivelis* appears to have diverged from an ancestor shared with *S. macrochir* about 4–5 mya. The ancestral distribution of the genus *Sebastolobus* (and the northern Pacific members of the sympatric pleuronectid *Microstomus*; Stepien 1995, 1999) may have once been continuous throughout the subarctic and cold temperate provinces across the northern Pacific Ocean. The northeastern Pacific lineage may have become allopatrically separated from the northwestern Pacific species due to changes in the distribution of oxygen minimum zones, i.e., the "deep sea allopatry hypothesis" (White 1987). Alternatively, the deep waters of the northern Aleutian Basin off the Siberian coast may have formed a significant barrier to east–west dispersal that persists to the present day (Briggs 1974). The present eastern and western faunas of the southern Bering Sea (below the subarctic zone) show marked separation, and approximately 67% of the taxa are distinct (summarized by Briggs 1974). A number of apparently geminate species pairs occur in the eastern and western North Pacific whose distributions mirror those of *Sebastolobus* and *Microstomus*, including members of the clam genus *Mya* and the fish families Cottidae, Liparidae, and Zoarcidae (Briggs 1974).

The phylogenetic analyses indicate that the ancestral haplotypes of *S. alascanus* and *S. altivelis* occupied opposing biogeographic provinces. The basal lineages of *S. alascanus* are currently restricted within the warm temperate province to a site off San Diego, while those of *S. altivelis* are found in the cold temperate province at sites in northern California and Oregon. This pattern suggests that their speciation, at the beginning of the Pleistocene (about 1.3 mya), was vicariant due to the formation of a temperature barrier of colder water (Briggs 1974). Because the present range of *S. alascanus* extends father north than that of *S. altivelis* (Eschmeyer et al. 1983; Jacobson and Vetter 1996), we can presume that the oceanographic range of the cold sensitive *S. altivelis* was more restricted during the Ice Ages. Based on our estimated mean sequence divergences between the ancestral lineages and the more derived groups within both species, further diversification occurred during the mid-Pleistocene, which (again) may be linked to temperature barriers. For example, the single clade of *S. altivelis* with clear phylogeographic structure includes haplotypes currently found only in neighboring sites within the northern area of the warm temperate province. By contrast, its two ancestral haplotypes (2 Oregon A, 1 N. Calif. A) are currently restricted to colder waters.

Levels of intraspecific genetic variability

In all three species of *Sebastolobus*, *h* within sampling locations, which is analogous to heterozygosity (Nei 1987), is high, averaging 0.99 in *S. alascanus*, 0.96 in *S. altivelis*, and 1.0 in *S. macrochir*. The *h* was similarly high (0.99) in the Dover sole, which is sympatric with *S. alascanus* and

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Table 1. Transversion haplotypes and their representation in the sampling locations for *Sebastolobus alascanus* and *S. altivelis*.

S. alascanus

	Nucleotide position															*N* per sample site							
	0	0	1	2	2	2	2	2	2	2	2	3	3	3	4	Bering	Cape	Cape	Monterey	Morro	Santa	San	
	1	3	1	0	0	0	3	5	6	9	9	0	2	3	4	Sea,	Arago,	Mend.,	Bay,	Bay,	Barbara,	Diego,	
Haplotype	5	3	5	2	4	6	1	9	7	0	7	4	6	5	2	Alaska	Oreg.	N. Calif.	C. Calif.	C. Calif.	S. Calif.	S. Calif.	Total
1	T	A	T	T	T	C	C	T	C	A	G	G	C	A	T	8	8	3	8	7	6	9	49
2	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	0	1	5	4	0	1	3	14
3	-	-	-	-	A	-	-	G	-	-	-	-	-	-	-	1	0	0	0	0	0	0	1
4	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	4	2	2	2	2	0	0	12
5	-	-	-	-	-	-	-	A	-	-	T	-	-	-	-	1	0	0	0	0	0	0	1
6	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	0	2	0	0	0	1	1	4
7	-	-	-	-	-	-	-	G	-	C	-	-	-	-	-	0	0	1	0	0	0	0	1
8	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	0	0	1	0	0	0	0	1
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	0	0	0	0	1	0	0	1
10	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	0	0	0	0	1	0	0	1
11	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	1	0	0	1
12	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	1	0	0	1
13	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	0	0	0	0	0	1	0	1
14	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	1	0	1
15	-	-	-	-	-	A	-	G	-	-	-	-	-	-	-	0	0	0	0	0	0	1	1
16	-	-	-	-	-	-	-	A	A	-	-	-	-	-	-	0	0	0	0	0	0	1	1
17	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	0	0	0	0	0	0	1	1
18	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0	1	1
Total																14	13	12	14	13	10	17	93

S. altivelis

	0	1	1	1	2	2	2	2	2	3	3	3	Cape	Cape	Morro	Santa	San	
	1	1	4	4	0	0	4	4	9	0	6	8	Arago,	Mend.,	Bay,	Barbara,	Diego,	
Haplotype	5	5	1	2	6	8	3	8	8	4	9	9	Oreg.	N. Calif.	Calif.	Calif.	Calif.	Total
1	T	T	C	A	T	C	C	T	A	G	T	G	5	13	11	3	9	41
2	-	-	-	-	-	-	-	-	-	-	A	-	1	0	0	0	0	1
3	G	-	-	-	-	-	-	-	-	-	-	-	0	1	0	0	0	1
4	-	-	-	-	A	-	-	-	-	-	-	-	0	1	0	0	0	1
5	-	-	-	-	-	G	-	-	-	-	-	-	0	1	0	0	0	1
6	-	A	-	-	-	-	-	-	-	-	-	-	0	0	1	0	0	1
7	-	-	G	T	-	-	-	-	-	T	-	-	0	0	0	1	0	1
8	-	-	G	T	-	-	-	-	-	-	-	-	0	0	0	1	0	1
9	-	-	-	T	-	-	-	-	-	-	-	-	0	0	0	2	0	2
10	-	-	-	T	-	-	-	A	-	-	-	-	0	0	0	1	0	1
11	-	-	-	T	-	-	A	-	-	-	-	-	0	0	0	1	0	1
12	-	-	-	-	-	-	-	-	C	-	-	-	0	0	0	0	1	1
13	-	-	-	-	-	-	-	-	-	-	-	T	0	0	0	0	2	2
Total													6	16	12	9	12	55

Note: Sample sites and their biogeographic provinces are shown in Fig. 1. Nucleotide positions refer to the aligned sequences given in the Appendix. Haplotypes are numbered. These data sets were used for the AMOVAs in Table 2.

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Table 2. AMOVAs (Excoffier 1995) for differences in the distribution of mtDNA control region transversion haplotypes (from Table 1) among pairs of sampling sites for *Sebastolobus alascanus* and *S. altivelis*.

S. alascanus

	Sample site (number)					
Sample site (number)	Seward, Bering Sea, Alaska (N = 14)	Cape Arago, Oreg. (N = 13)	Cape Mendocino, N. Calif. (N = 12)	Monterey Bay, C. Calif. (N = 14)	Morro Bay, C. Calif. (N = 13)	Santa Barbara, S. Calif. (N = 10)
Oregon	0.020					
	$P < 0.225$					
Northern California	0.050	0.127				
	$P < 0.179$	$P < 0.032$*				
Monterey Bay	0.004	0.025	0.008			
	$P < 0.385$	$P < 0.202$	$P < 0.427$			
Morro Bay	0.052	0.019	0.159	0.078		
	$P < 0.048$*	$P < 0.405$	$P < 0.001$**	$P < 0.056$		
Santa Barbara	0.093	0.030	0.176	0.070	0.021	
	$P < 0.024$*	$P < 0.702$	$P < 0.001$**	$P < 0.089$	$P < 0.482$	
San Diego (N = 17)	0.034	0.035	0.105	0.021	0.045	0.017
	$P < 0.125$	$P < 0.814$	$P < 0.001$**	$P < 0.640$	$P < 0.062$	$P < 0.062$

S. altivelis

	Sample site (number)			
Sample site (number)	Cape Arago, Oreg. (N = 6)	Cape Mendocino, N. Calif. (N = 16)	Morro Bay, C. Calif. (N = 12)	Santa Barbara, S. Calif. (N = 9)
Northern California	0.017			
	$P < 0.653$			
Morro Bay	0.033	0.004		
	$P < 0.102$	$P < 0.842$		
Santa Barbara	0.304	0.430	0.412	
	$P < 0.039$*	$P < 0.001$**	$P < 0.001$**	
San Diego (N = 12)	0.021	0.049	0.046	0.362
	$P < 0.489$	$P < 0.171$	$P < 0.171$	$P < 0.001$**

Note: Values indicate ϕ_{ST} and the probability (P) of obtaining a random number greater than the value from 1000 permutations. *Significant difference at $P < 0.05$; **significant difference at $P < 0.05/N$ pairwise comparisons using a Bonferroni correction for multiple tests (Sokal and Rohlf 1995). For *S. alascanus*: variance among biogeographic provinces = 1.3% (not significant); among sample sites within biogeographic provinces = 4.4%, ϕ_{ST} = 0.064, $P < 0.033$*; within sites = 94.3%, ϕ_{ST} = 0.020, $P < 0.020$*. For *S. altivelis*: variance among biogeographic provinces = 3.8% (not significant); among sites within biogeographic provinces = 24.0%, ϕ_{ST} = 0.249, $P < 0.001$*; within sites = 72.2%, ϕ_{ST} = 0.278, $P < 0.001$*.

S. altivelis along the deep continental slopes of the northeastern Pacific. However, levels of π within sampling sites are moderate (Nei 1987; Bowen and Grant 1997), averaging 0.015 for *S. alascanus*, 0.009 for *S. altivelis*, and 0.016 for *S. macrochir*. Levels of π also are comparable with those of the Dover sole (averaging 0.014; Stepien 1999). Similarly, Bowen and Grant (1997) and Grant and Bowen (1998) discerned high h and moderate to low π in mtDNA left domain control region sequences of sardine and anchovy populations, which are pelagic in surface waters throughout their life histories and are much more r-selected than continental slope fishes (Eschmeyer et al. 1983). High h suggests large, relatively stable effective population sizes over time (Nei 1987) in the continental slope fishes. The contrasting values for h and π describe populations that contain a large number of closely related haplotypes (Bowen and Grant 1997), as indicated in the MP and NJ trees for the thornyheads.

Gene flow and phylogeographic relationships among populations

Nucleotide divergences among sampling sites are much greater for *S. altivelis* than for *S. alascanus*, suggesting less gene flow in the former. This interspecific difference can be attributed to their differences in migration as juveniles and adults (Wakefield 1990). By comparison, divergences among samples of *M. pacificus* were intermediate (0.0010 ± 0.0001), indicating some significant geographic structuring (Stepien 1999). By comparison, genetic divergences among populations of *M. pacificus* were not related to geographic distances and appeared to be consistent with the larval retention hypothesis (Stepien 1999).

Significant differences in the distributions of haplotypes among some sampling sites for *S. alascanus* and *S. altivelis* also indicate barriers to gene flow. Significant population genetic structure is discerned in our study that was undetected with allozymes by Siebenaller (1978), due to the greater resolution power of sequencing the highly variable mtDNA control region (Stepien 1995). Relationships among populations of *S. alascanus* fit an isolation by geographic distance pattern. In contrast, population genetic divergences of *S. altivelis* are not related to geographic distances and suggest larval retention (this study; Pearcy et al. 1977). Geo-

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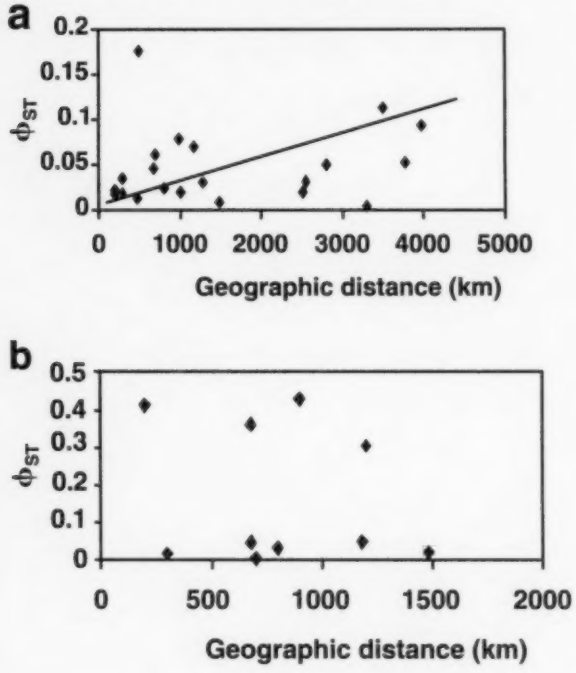
Fig. 3. Plot of ϕ_{ST} values versus geographical distances between pairs of sampling sites for (*a*) *S. alascanus* (correlation r = 0.184. Explained (1 df) SS = 0.001, MS = 0.001; residual (16 df) SS = 0.031, MS = 0.002, F = 0.56, P = 0.047 (significant with $\alpha < 0.05$)) and (*b*) *S. altivelis* (correlation r = –0.16. Explained (1 df) SS = 0.04, MS = 0.04; residual (7 df) SS = 0.24, MS = 0.03, F = 1.19, P = 0.62 (not significant)).



graphic relationships and patterns among populations of continental slope fishes thus differ for shortspine thornyhead, longspine thornyhead, and Dover sole (Stepien 1999).

Longspine thornyhead from the Santa Barbara site form a geographically circumscribed cluster of haplotypes, united by a transversional synapomorphy at nucleotide position 142, that differ significantly from those in other locations. This genetic divergence can be explained by the more restricted movements of juvenile *S. altivelis* that "settle out" in adult benthic habitats at a younger age than *S. alascanus* (Wakefield 1990; Jacobson and Vetter 1996). The longer midwater larval period of *S. altivelis* also may transport them further northward in the California Countercurrent, compensating for earlier southward transport in surface waters (Butler et al. 1996). This genetic divergence of *S. altivelis* in the Santa Barbara region should be further tested with larger sample sizes. This geographically circumscribed group of *S. altivelis* may be particularly important for maintaining the genetic diversity and preserving the phylogeographic history of the species.

The member/vagrant hypothesis of larval retention (Sinclair 1988) appears to be consistent with some of the present results. The distribution of longspine thornyhead haplotypes, most notably in the Santa Barbara region, indicates barriers to larval dispersal between currents and offshore gyres that limit gene flow. Some population areas may "lose" more larvae to southerly transport via the California Current. Further studies are needed to examine the relationship of current patterns to the genetics of larval year-classes.

Alternatively, these genetic patterns may be regulated by temperature-related selection on larval survival. Surface water temperatures differ significantly among the primary biogeographic regions, and distributions of marine fish larvae appear to be sensitive to such boundaries (see Stepien 1995; Grant and Bowen 1998). However, the primary phylogeographic divisions occur among individual sampling sites in our data sets and do not appear to correspond to the surface temperature biogeographic provinces. Populations of *S. altivelis* located in the same biogeographic province and (or) in close proximity are not necessarily more closely related, and divergences differ from those of *S. alascanus* (this study) and *M. pacificus* (Stepien 1999). Nevertheless, this alternative hypothesis may be tested further by comparing genetic characters between early larval stages in the surface waters versus later larval and juvenile stages in deeper waters and measuring their relationships to adult populations. If thornyheads are panmictic during their early life history stages, but display significantly different distributions in later stages, then genetic patterns may be due to selective survival related to the member/vagrant hypothesis of retention (Sinclair 1988). The number of population genetic units may be equivalent to the number of discrete larval retention areas over the species' range (Sinclair 1988).

mtDNA sequences from the control region of the sympatric *S. alascanus* and *S. altivelis* in the present study and *M. pacificus* (Stepien 1999) showed similarly high variability but different phylogeographic relationships among populations. Differences in the geographic genetic structures among the three species suggest that different mechanisms are involved in each case, with variations in migration patterns of juveniles and adults (Wakefield 1990), differential retention of larvae in currents (Sinclair 1988), and (or) selection factors. In contrast with the present results, another continental slope species, orange roughy (*Hoplostethus atlanticus*), showed little population divergence in allozymes, mtDNA RFLPs, and nuclear RAPD markers across its wide range (Smith et al. 1997). *Hoplostethus atlanticus* may have lower larval retention, in contrast with the northeastern Pacific slope fishes. More similar to the present study, Wilson and Waples (1984) discerned geographic patterns (suggesting subspecies-level separation) from allozyme data among populations of the deep-sea abyssal grenadier (*Coryphaenoides armatus*), which also has pelagic larvae. All together, these studies suggest that life history factors, larval retention, and recruitment produce differential geographic genetic structure in deep-sea fishes that have pelagic early life history stages.

In conclusion, the thornyhead populations of the northern Pacific continental slopes are heavily exploited by trawling (Gunderson 1997), rendering data on their genetic variability and population structures critically important. The present investigation and the companion study on the Dover sole (Stepien 1999) of mtDNA control region sequences show greater phylogeographic structure along deep continental slopes than was suspected previously (Siebenaller 1978; Stepien 1995; Smith et al. 1997). Some of these results are consistent with the member/vagrant hypothesis (Sinclair

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1988) of pelagic larval retention in currents and gyres by species that are benthic as adults, including the longspine thornyhead (this study) and the Dover sole (Stepien 1999). In contrast, populations of the shortspine thornyhead reveal a pattern of genetic divergence corresponding to geographic distance. Current and gyre patterns, larval behavior, and successful recruitment near adult populations thus may produce differential phylogeographic patterns in deep-sea regions. Results of the present study indicate that marine populations that have been regarded as genetically homogenous, such as those in deep-sea regions, may house considerable structure. Life history factors, notably duration of pelagic dispersal stages, may produce marked differences in population genetic structure between closely related sympatric species.

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Appendix. Summarized sequence data for the mtDNA control region of *Sebastolobus alascanus*, *S. altivelis*, and *S. macrochir* aligned with sequences for the outgroup *Sebastes marinus* (from Lee et al. 1995).

	Base position																							
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4
S. alascanus	T	C	A	A	A	G	A	A	A	G	G	A	G	A	T G	T	T	T	A	A	C	T	C	C
S. altivelis	T	C	A	A	A	G	A	A	A	G	G	A	G	A	T G	T	T	T	A	A	C	T	C	C
S. macrochir	T	C	A	A	A	G	A	A	A	G	G	A	G	A	T	T	T	T	A	A	C	T	C	C
S. marinus	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Base position																							
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4
S. alascanus	C	T	G	A	G	T	T	A	A	A	C	T	A	T	T	C	T	T	T	G	T	A	T	-
S. altivelis	C	T	G	A	G	T	T	A	A	A G	C	T	A	T	T	C	T	T	T	G	T	A	T	-
S. macrochir	C	T	G	A	G	T	T	A	A	A	C	T	A	T	T	C	T	T	T	G	T	A	T	-
S. marinus	C	T	T f	A	G	T	T	A	A	A	C	T	A	T	T	C	T	T	T	G	T	A	T	G f
	Base position																							
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4
S. alascanus	T	G	T	A	T	T	A	T	C	A	C	C	A	T	T A	A	A	T	T	T	A	T	A	T
S. altivelis	T	G	T	A	T	T	A	T	C	A	C	C	A	T	T A	A	A	T	T	T	A	T	A	T
S. macrochir	T	G	T A	A	T	T	A	T	C	A	C	C	A	T	T	A	A	T	T	T	A	T	A	T
S. marinus	T	G	T	A	T	T	A	T	C	A	C	C	A	T	T	A [	A t a	T e s	T r s	T m o	A i c.	T n	A a	T t s
	Base position																							
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4
S. alascanus	C	A	T	A	C T	A	T	G	T C	T	T	T C	A G	T C	C T	C	A	C	A	T C	A	T C	G	T
S. altivelis	C	A	T	A	C	A	T	G	T	T	T	T	A	T	C	C	A	C	A	T	A	T	G	T

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0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	5
5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0
C	A	C	C	C	C	T	G	G	C	T	C	C	C	A	A	A	G	C	C	A	G	G	A	T	T
								C																	
C	A	C	C	C	C	T	G	G	C	T	C	C	C	A	A	A	G	C	C	A	G	G	A	T	T
C	A	C	C	C	C	T	G	G	C	T	C	C	C	A	A	A	G	C	C	A	G	G	A	T	T
N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	C	C	A	G	G	A	T	T

0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
7	7	7	7	7	8	8	8	8	8	8	8	8	8	8	9	9	9	9	9	9	9	9	9	9	0
5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0
-	-	-	-	-	-	-	A	A	T	A	T	A	-	-	-	-	A	T	A	C	A	T	G	T	A
-	-	-	-	-	-	-	A	A	T	A	T	A	-	-	-	-	A	T	A	C	A	T	G	T	A
								G																	
-	-	-	-	-	-	-	A	A	T	A	T	A	-	-	-	-	A	T	A	C	A	T	G	T	A
T	A	T	G	T	A	C	A	A	T	A	T	T	T	C	A	T	A	T	A	C	A	T	A	T	A
f	f	f	f	f	f	f							f	f	f	f							f		

1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	5
5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0
T	A	A	C	C	A	T	A	T	C	A	A	T	A	-	G	C	A	T	T	C	A	A	G	T	A
																					G				
T	A	A	C	C	A	T	A	T	C	A	A	T	A	-	G	C	A	T	T	C	A	A	G	T	A
																G	T								
T	A	A	C	C	AT	T	A	T	C	A	A	T	A	-	G	C	A	A	T	C	A	A	G	T	A
																		f							
T	A	A	C	C	A	T	A	T	C	A	T	A	G	G	G	C	A	T	T	C	A	T	A	T	A
i	o	n										f	f	f								f	f		
e	q				]																				

1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
7	7	7	7	7	8	8	8	8	8	8	8	8	8	8	9	9	9	9	9	9	9	9	9	9	0
5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0
A	G	G	T	T	T	T	A	A	G	C	A	T	T	C	A	C	T	T	A	T	C	A	A	C	-
G					C				A							T			G						
A	G	G	T	T	T	T	A	A	G	C	A	T	T	C	A	C	T	T	A	T	C	A	A	C	-

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Appendix (*continued*).

	Base position																								
	15 51	15 52	15 53	15 54	15 55	15 56	15 57	15 58	15 59	16 00	16 01	16 02	16 03	16 04	16 05	16 06	16 07	16 08	16 09	16 10	17 11	17 12	17 13	17 14
S. macrochir	C	A	T	A	T T C s	A	T	G	T	T	T	T	A	T	T C	C	A	C T	A	T	A	T	G	T
S. marinus	C	A	T	A	T	A	T	G	T	A f	T	T	A	T	C	A f	C f	C	A	T	A	T	A f	T

	Base position																								
	20 01	20 02	20 03	20 04	20 05	20 06	20 07	20 08	20 09	20 10	21 11	21 12	21 13	21 14	21 15	21 16	21 17	21 18	21 19	22 20	22 21	22 22	22 23	22 24
S. alascanus	A	T C A	A G	T C A	A G	C T A	T C	T C	A	A	G	A	T	A	-	T	C T	C	A	T	A G	A	A	G
S. altivelis	A	T C	A	T	A	T C A s	T	C T G s	A	A	G A	A	T	A	-	T	C	C	A	T	A	A	A	G
S. macrochir	A	T C	A	A s	A	A s	C s	T s	A	A	T C s	A G	T	A	-	T	A f	C	A	C f	A G	A G	A	G
S. marinus	A	C s	G s	A	C f	A	C	G s	A	A	C s	A	T	T f	T f	T	A	C	A	T	A	A	A	G

	Base position																								
	25 51	25 52	25 53	25 54	25 55	25 56	25 57	25 58	25 59	26 00	26 01	26 02	26 03	26 04	26 05	26 06	26 07	26 08	26 09	26 10	27 11	27 12	27 13	27 14
S. alascanus	A	-	T	T C	A	C	A	T	T C G A	A	A	A G	C	A	G	G	C A	G	A	A	A	C T	T	T
S. altivelis	A G	-	T	T C	A	C	A	T C	C T	A	A	A G	C	A	G	G	C	G	A	A	A	C T	T	T
S. macrochir	G A s	A f	T	T A	A G	T	G A s	T	G s	A	A	A G	C	A	G	G	C	G	A	A	A	G f	T	C f
S. marinus	- f	-	T	T	A	T f	A s	A	A f	T s	A f	C s	C	A	A f	G	C	G	A	A	A	T	T	T

	Base position																								
	30 01	30 02	30 03	30 04	30 05	30 06	30 07	30 08	30 09	30 10	31 11	31 12	31 13	31 14	31 15	31 16	31 17	31 18	31 19	32 20	32 21	32 22	32 23	32 24
S. alascanus	T C	A	A	G T	T	T	A	T	A	C	C	T	T	T	A	C	T	C	A	A	A	C T	T	C
S. altivelis	T C	A	A	G T	T	T	A	T	A	C	C	T	T	T	A	C T	T	C	A	A	A	C T	T	C
S. macrochir	C s	A	A	G	T	T	A	T G	A T	C	C	T	T	T	A	C	T	C	A	A	A	C	T	C
S. marinus	T	A	A	G	T	T	A	T	A	C	C	T	T	T	A	C	T	C	A	A	A	A	T	C

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175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200
AC	G	G	T	T	T	T	A	A	AG	C	A	CT	T	C	A	C	T	T	GG	T	C	A	A	C	-
A	G	A	A	T	T	T	A	A	C	C	A	T	T	C	A	C	A f	A f	sG	T f		T f	T f	A f	T f

225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250
C	A	TC	G	AG	AG	CTGCT	TC	T	A	T	AG	-	-	TC	A	AG	TC	CT	A	CT	A	AG	T	AG	A
C	A	T	G	AG	AG	CT	T	T	A	T	AG	-	-	T	AG	AG	CT	CTA	A	TC	A	AG	TA	AG	AG
C	A	T	G	A	A	CT	T	C f	A	T	A	-	-	T	A	A	C	A f	A	sCT	A	A	T	A	A
T	A	A f	A f		T f	A f	A f	T	A	A f	G	A f	C f	T	A	A	C	A	A	A f	C	A	C f	- f	- f

275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300
A	A	G	A	C	C	T	A	A	C	A	C	A	AG	T	AC	A	AG	TC		A	TC	GTA	AG	G	T
A	A	G	A	C	C	T	A	A	C	A	C	A	A	T	A	A	A	T	C	A	T	AG	ACG	G	T
A	A	G	AG	C	C	T	A	A	C	A	C	A	AG	T	A	A	A	T	C	AG	T	A	A	G	T
A	A	G	A	C	C	G f	A	A	C	A	C	A	A	A f	C s	A	C f	T	C	A	T	A	A	G	T

325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350
TC	CGT C	G	TC	CT	A	A	T	T	T	AGC	A	AG	A	A	T	CT	T	T	A	A	T	G	T	A	G
T	C	G	CTs	C	A	A	T	T	T	A	A	A	A	A	T	C	T	T	A	A	T	G	T	A	G
CT	C	G	T	C	A	A	T	T	T	A	A	A	A	A	T	C	T	T	A	A	T	G	T	A	G
C	Ts	A f	T	C	A	A	A f	C f	T	Cs	A	A	A	T f	A f	Ts	T	T	A	A	G f	A f	G f	C f	C f

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Appendix (*concluded*).

	Base position																							
	35151	35152	35153	35154	35155	35156	35157	35158	35159	36160	36161	36162	36163	36164	36165	36166	36167	36168	36169	37170	37171	37172	37173	37174
S. alascanus	T	A	A	G	A	G	C	C	G	A	C	C	A	A	C T	A	A	G	T	C	C	A	T	T
S. altivelis	T	A	A	G	A	G	C	C	G A	A	C	C	A	A	C	A	A	G	T A	C	C	A G	T	T
S. macrochir	T	A	A	G	A T	G	C	C	G	A	C	C	A	A	C	A	A	G	T	C	C	A	T	T
S. marinus	G f	A	A	T f	G f	T f	A f	G f	T f	A	C	C	A	A	C	A	A	G	T	C	C	A	T	T
	Base position																							
	40161	40162	40163	40164	40165	40166	40167	40168	40169	41170	41171	41172	41173	41174	41175	41176	41177	41178	41179	42180	42181	42182	42183	42184
S. alascanus	T	G	A	G	G	G	-	A	C	A	A	T	G	A	T	T	C	G	T	G	G	G	G	G
S. altivelis	T	G	A	G	G	G	G f	A	C	A	A	T	G	A	T	T	C T	G	T	G	G	G	G	G
S. macrochir	T	G	A	G	G	G	-	A	C	A	A	C T	A f	A	T	T	C	G	T	G	G	G	G	G
S. marinus	T c	G o	A n	G s	G e	G r	- v	A e	C d	A	A	T	T f s	A e	T c	T t	- f i	G o	T n	G -	G -	G -	G -	G -
	Base position																							
	45161	45162	45163	45164	45165	45166	45167	45168	45169	46170	46171	46172	46173											
S. alascanus	T	C	T	G	G	T	T	C	C	T	A	C	T											
S. altivelis	T	C	T	G	G	T	T	C	C	T	A	C	T											
S. macrochir	T	C	T	G	G	T	T	C	C	T	A	C	T											
S. marinus	T s	C e	T q	G	N b	N l	N o	N c	N k	N D	N]	N	N											

Note: Numbers correspond to nucleotide positions. Substitutions are indicated, in order of descending relative frequency, below the consensus (most common) nucleotides in the species. Structural features of the mtDNA control region, shared with other vertebrates, are designated below the sequences. "N" represents nucleotide position not sequenced by Lee et al. (Lee, W.J., Conroy, J., Howell, W.H., and Kocher, T.D. 1995. Structure and evolution of teleost mitochondrial control regions. J. Mol. Evol. **41**: 54–66) for the outgroup, "f" represents fixed difference in base composition among the species, and "s" represents significant difference among the species in nucleotide frequencies from contingency table or chi-square tests at $P < 0.05$ (Sokal, R.R., and Rohlf, F.J. 1995. Biometry. 3rd ed. W.H. Freeman and Co., San Francisco, Calif.).

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3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4
7	7	7	7	7	8	8	8	8	8	8	8	8	8	8	9	9	9	9	9	9	9	9	9	0
5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0
T	C	T	T	A	A	T	G	C	C	A	A	C	G	G	T	T	A	T	T	G	A	A	G	G	-
									T													G			
T	C	T	T	A	A	T	G	C	C	A	A	C	G	G	T	T	A	T	T	G	A	A	G	G	G
														T											
T	C	T	T	A	A	T	G	C	C	A	A	C	G	G	T	T	A	T	T	G	A	A	G	G	f
C	-	T	T	A	A	T	G	T	C	A	A	C	G	G	T	T	A	T	T	G	A	A	G	G	-
f	f							f									[	c	e	n	t	r	a	l	

4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4
5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0
T	T	T	C	A	C	A	C	A	G	T	G	A	A	C	T	A	T	T	C	C	T	G	G	C	A
C						G								T		G	A								
T	T	T	C	A	C	A	C	-	G	T	G	A	A	C	T	A	T	T	C	C	T	G	G	C	A
								f															A		
T	T	A	C	A	C	G	A	-	G	T	G	A	A	C	T	A	T	T	C	C	T	G	G	C	A
						s	G	f																	
T	T	T	C	A	C	A	C	A	G	T	G	A	T	T	T	A	T	T	C	C	T	G	G	C	A
		f											f	f											
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	]	[	-	-	c	o	n	s.		

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Gillnet selectivity and size and age structure of an alpine Arctic char (*Salvelinus alpinus*) population

Anders G. Finstad, Peder A. Jansen, and Arnfinn Langeland

Abstract: The aim of the present study was to address possible implications of biased sampling for the commonly adopted uni- and bi-modal size structures and unimodal age structures in Arctic char (*Salvelinus alpinus*) populations. Multimesh gill nets were used to sample an allopatric population of Arctic char in an alpine lake in central Norway. Direct estimates of gillnet selectivity for different length-classes of Arctic char were obtained by mark–recapture experiments and by successive removal methods. The observed size and age structure in gillnet samples was significantly different from the estimated size and age structure of the Arctic char population when catches were corrected for gillnet selectivity. An observed unimodal size and age structure was a direct result of gillnet selectivity, as smaller and younger fish were underrepresented in gillnet catches. Moreover, an abrupt increase in gillnet selectivity for large Arctic char was related to a niche shift to cannibalism. A model that explains bimodal size distributions in gillnet catches as a result of ontogenetic behavioural change is presented. Complex ontogenetic growth and mortality patterns that have been suggested to be essential in structuring modal Arctic char populations were superfluous in explaining the observed modal population structure in the present study.

Résumé : L'objet de la présente étude était d'examiner les incidences possibles du biais dans l'échantillonnage sur les structures de taille uni- et bi-modales et les structures d'âge unimodales dans les populations d'omble chevalier. Nous avons utilisé des filets maillants à maillages multiples pour échantillonner une population allopatrique d'omble chevalier (*Salvelinus alpinus*) dans un lac alpin du centre de la Norvège. Les estimations directes de la sélectivité des filets maillants pour différentes classes de longueurs ont été obtenues par des expériences de marquage–recapture et par des méthodes de prélèvements successifs. La structure de taille et d'âge observée dans les échantillons prélevés au filet maillant était significativement différente de la structure estimée lorsqu'on corrigeait les captures pour tenir compte de la sélectivité du filet maillant. La structure unimodale de taille et d'âge observée était directement attribuable à la sélectivité du filet maillant, car les poissons petits et jeunes étaient sous-représentés dans les captures. De plus, une augmentation brutale de la sélectivité du filet maillant pour les ombles de grosse taille était en rapport avec un changement de niche avec passage au cannibalisme. Nous présentons un modèle qui attribue les distributions bimodales de taille dans les captures au filet maillant à un changement de comportement ontogénétique. Les patrons ontogénétiques complexes de croissance et de mortalité qui étaient proposés comme essentiels dans la structuration des populations modales d'ombles chevaliers ont été inutiles pour expliquer la structure modale observée dans la population qui était l'objet de notre étude.

[Traduit par la Rédaction]

Introduction

Size distributions of unexploited Arctic char (*Salvelinus alpinus*) populations are commonly reported to be unimodal or bimodal and age structures unimodal and bell-shaped (Johnson 1976; Griffiths 1994; Svenning and Borgstrøm 1995). Modal population structure is characterised by a high degree of uniformity in size, a high mean age, and few recruits. The lower length mode of bimodal Arctic char populations commonly resembles the size distribution of unimodal populations (Johnson 1976; Sparholt 1985; Parker and Johnson 1991). Similar uni- and bi-modal population structures are also observed in Arctic populations of lake whitefish (*Coregonus clupeaformis*) and lake trout (*Salvelinus namaycush*) (Johnson 1976). Bell-shaped age structures in Arctic freshwater fish populations are reported to persist over decades (Johnson 1976). This excludes variable intercohort recruitment as the founding process for such age structures. Johnson (1976) proposed that the underlying mechanisms structuring unexploited northern stocks of freshwater fish require a complex ontogenetic growth and mortality pattern. He hypothesised that recruitment is regulated by adult suppression of juveniles in the main part of the habitat, which is occupied by the modal size-class consisting of adult fish. An opportunity for the peripherally located and suppressed fish to move into more profitable parts of the habitat only arises after displacement of adult fish. These recruits then grow rapidly to quickly approach the modal size.

Power (1978) argued that modal size and age structures in northern populations of lake trout and lake whitefish may reflect an underestimation of small fish in combination with erroneous age determination. Assuming such underestimation, high growth and mortality rates in the juvenile fish, combined with gradually decreasing growth and mortality

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A.G. Finstad,[1] P.A. Jansen, and A. Langeland. Department of Zoology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway.

[1]Author to whom all correspondence should be addressed.
e-mail: anders.finstad@chembio.ntnu.no

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rates for the rest of the life span, may explain the origin of bimodal or unimodal size distributions. Nevertheless, modal patterns in Arctic char are commonly associated with Johnson's (1976) hypothesis (Sparholt 1985; Parker and Johnson 1991; Gullestad and Klemetsen 1997).

Obtaining representative samples of all size- and age-classes is of vital importance when assessing within- and among-population interactions. When size distribution and demographic structure are unknown, the sampling method has to be nonselective with regard to age and size, or the selectivity of the sampling gear must be known. A gill net of a specific mesh size is selective with regard to fish size (Hamley 1975), and a common method to obtain representative samples from fish populations is to combine gill nets of various mesh sizes or to compose a net of several segments of different mesh sizes. The size distribution of gillnet samples will only be representative if the pooled efficiency of the combination of mesh sizes in use is equal for each size-class present in the population. Assessment of gillnet selectivity and the pooled efficiency of combinations of mesh sizes is commonly based on indirect methods (K.W. Jensen 1977; J.W. Jensen 1990; Appelberg et al. 1995). These methods, which are based on comparing catches obtained by different mesh sizes, assume the relative efficiency for all mesh sizes to be equal (Hamley 1975). However, direct estimates of gillnet selectivity, based on known population structures, ordinarily show that the efficiency of the net increases with increasing mesh size (e.g., Hamley 1975; Jensen 1995*a*; Jensen and Hesthagen 1996), implying that the assumptions of the indirect methods do not hold.

Although gillnet sampling is a widely used sampling method, information regarding length-class variation in gillnet selectivity, as well as its causes and consequences for interpretation of population structure, is often neglected. The purpose of the present study was to test if the commonly observed uni- and bi-modal size structures and unimodal age structures in Arctic char populations may be a result of the sampling procedure. The size and age structure of an alpine Arctic char population, obtained through multimesh gillnet samples, was compared with size and age structure derived from direct estimates of gillnet selectivity. Direct estimates of gillnet selectivity for length-classes 60–179 mm were provided using mark–recapture methods in a stratified sampling procedure. Gillnet selectivity for large Arctic char (>250 mm) was estimated in a separate experiment involving a mark–recapture experiment in combination with successive removal of large fish. The resulting size and age structure of the population is assessed with respect to Johnson's (1976) hypothesis regarding patterns of ontogenetic growth and mortality affecting size and age structures of Arctic fish populations.

Materials and methods

Study site

The study was carried out in Øvre Skarddørsjø (62°51′N, 12°05′E), central Norway (Fig. 1). Øvre Skarddørsjø is an ultraoligothrophic monomictic alpine lake 1118 m above sea level. The surface area is 42 ha, maximum depth is 32 m, and Secchi disc transparency is 18 m in summer. The ice-free season is restricted to July to late October and water temperatures then range from 1.5 to 12°C. The morphometry of the lake is relatively uniform (Fig. 1) and the bottom substrate consists of gravel, stone, and fine sand. The shoreline is homogeneous and consists of gravel and stone. The lake holds a stationary allopatric population of Arctic char and piscivory is frequent in the largest fish (Schartau et al. 1994). Due to a remote geographical location, fishing activity is negligible.

Sampling and treatment of material

Sampling with gill nets was conducted during July and August 1997 and June to September 1998 (Table 1). The multimesh gill nets used were monofilament bottom gill nets (1.5 × 30 m) of a type proposed for monitoring use in Nordic countries (referred to as NORDIC gill nets) (Appelberg et al. 1995). The net is composed of 12 mesh sizes (monofilament thickness, millimetres): 5 (0.10), 6.25 (0.10), 8 (0.10), 10 (0.13), 12.5 (0.13), 15.5 (0.15), 19.5 (0.15), 24 (0.15), 29 (0.15), 35 (0.16), 43 (0.20), and 55 (0.23) mm (bar mesh size). In addition, single mesh size bottom gill nets (1.5 × 10 m) with mesh sizes 5, 8, 10, 12.5, and 14.5 mm and single mesh size bottom gill nets (1.5 × 30 m) with mesh sizes 19.5, 24, 29, 35, 40, and 45 mm were used. Except for gill nets used in mark–recapture experiment 1 and gill nets used in estimating gillnet selectivity in the estimation area (see below), all gill nets were set perpendicular from the shore down to a maximum depth of 32 m at random throughout the lake. Previous sampling with pelagic gill nets (Schartau et al. 1994) gave only insignificant catches. Epibenthic habitat use was also confirmed in the present study by echosounder surveys during the sampling periods. Pelagic gill nets were therefore omitted. In addition, fish were sampled with electric fishing gear (pulsed DC back packer, current output 650 V at 1000 Ω, pulse length 1.8 ms at 70 Hz). Using an anode mounted on a 2-m rod, we caught fish down to a depth of approximately 1.5 m. Finally, baited funnel traps (12 with mesh size 10 mm and eight with mesh size 5 mm) were used to catch fish in 1998.

Total fish length (to the nearest millimetre) was measured with the tail spread in a natural position for all fish. Fish sampled with NORDIC gill nets and a subsample of fish caught with electric fishing gear were weighed to the nearest gram and aged by means of sacculus otoliths according to Nordeng (1961). The occurrence of fish in stomach contents was registered.

Assessment of gillnet selectivity

A direct estimate of pooled relative size selectivity (see Hamley 1975) (hereafter referred to as relative selectivity) for the NORDIC gill net was obtained using a stratified sampling procedure (see Bohlin et al. 1989) for the length-classes 60–89, 90–119, 120–149, and 150–179 mm in August 1997 by (*i*) estimating the frequency of each length-class in an estimation area (depth approximately 0–3 m, length approximately 1000 m) (Fig. 1) and (*ii*) assessing the frequency of the same length-classes in NORDIC gill nets in the same area and period.

The relative selectivity for the length-class >250 mm was obtained in a separate experiment by (*i*) estimating the frequency of fish >250 mm in the total population by comparing a successive removal estimate of fish >250 mm conducted during 1997 and 1998 with an estimate of the total number of fish in the population in length-classes 60–179 mm achieved in 1998 and (*ii*) assessing the frequency of the length-class >250 mm in NORDIC gillnet samples from the whole lake in 1998.

It was not possible to obtain a reliable estimate of the absolute or relative abundance, and hence selectivity, for length-classes 180–249 mm.

Selectivity for length-classes 60–179 mm (mark–recapture experiment 1)

Fish were caught with electric fishing gear in the shallow part of the estimation area and marked with removal of the pelvic and adipose fins in different combinations. The fish were observed for 10–30 min after marking. Out of 1266 fish marked, 31 fish were found

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Fig. 1. Study area, Øvre Skarddørsjø, central Norway (62°51′N, 12°05′E). Thin lines indicate 5- m depth contours, the grey area indicates the estimation area (mark–recapture experiment 1), broken lines indicate the location of gill nets used for recapture in mark–recapture experiment 2, and F indicates the location of funnel traps used in catching fish for marking in 1998.

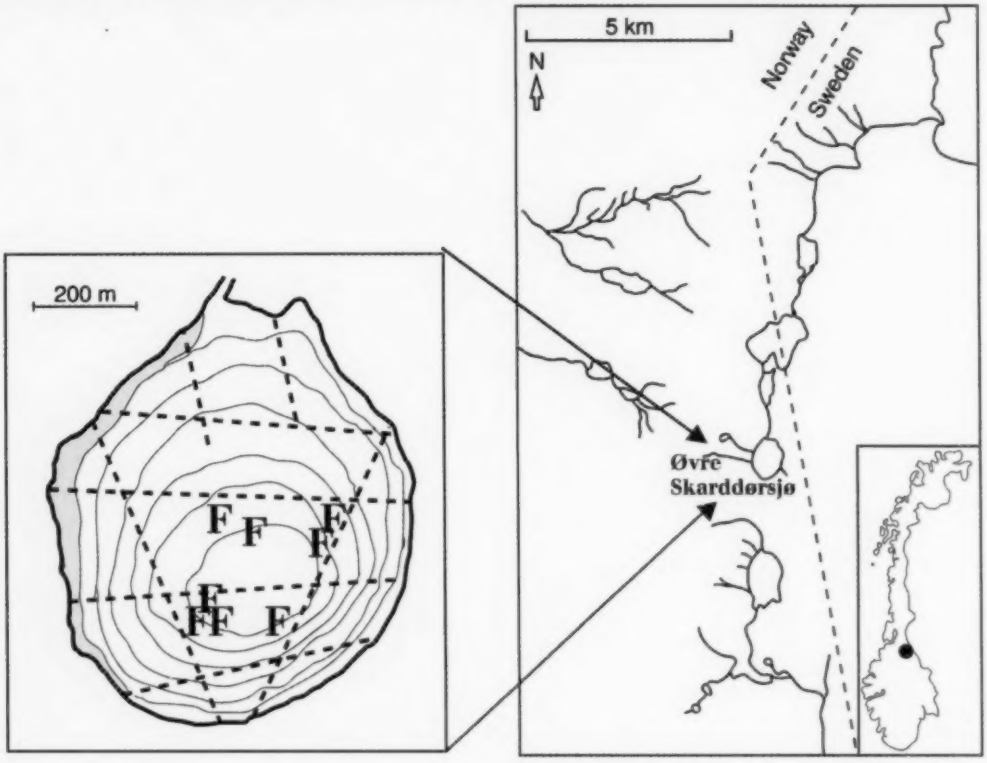

Table 1. Number of Arctic char captured with NORDIC and single mesh size gill nets (gillnet effort is given in parentheses as the number of gillnet nights) and number of fish caught with electric fishing gear and funnel traps and later released (with the number sampled for age determination in parentheses).

Date	NORDIC gill nets	Single gill nets		Electric fishing gear	Funnel traps
		1.5 × 30 m	1.5 × 10 m		
July 1997	164 (16)	5 (6)			
August 1997	262 (16)		661 (40)	2554 (120)	
June 1998	22 (8)				
August 1998	262 (30)	56 (12)	19 (7)	87 (87)	1557
September 1998	142 (40)	35 (10)	155 (26)	39 (39)	167

not to be in an acceptable state for release and were killed. To control for migration, the estimation area was divided into 11 different subsections of approximately equal size and fish were marked in seven of the different subsections with a specific combination of removed fins. The rest of the subsections were used for recapture only. Additional control for migration was achieved by sampling the rest of the lake with gill nets in the same period. The fish were released at random in the same subsection as they were captured. The first marking of fish was carried out during August 2–4, 1997, the second marking and the first recapture during August 7 and 8, 1997, and the final recapture during August 19–21, 1997. In addition to electric fishing gear, single mesh size gill nets (1.5 × 10 m) with mesh sizes 5, 8, 10, 12.5, and 14.5 mm, set randomly at depths from 0 to 3 m, were used at the final recapture. The fishing effort was evenly distributed throughout the estimation area in all periods. The estimated number of fish in each length-class (±95% CI) was calculated according to Chapman's modification of Schnabel's estimate (see Ricker 1975). Length-classes were set to 30 mm, which gave an acceptable precision of the estimates.

Relative selectivity for each length-class was calculated as the frequency of the length-class in catches from NORDIC gill nets set at random in the estimation area divided by the estimated frequency of the length-class from mark–recapture experiment 1.

Selectivity for the length-class >250 mm (successive removal experiment in combination with mark–recapture experiment 2)

The successive removal estimate of fish >250 mm was obtained through linear regression of catch per unit of effort (CPUE) against cumulative catch from July 1997 to October 1998 following Ricker (1975). NORDIC gill nets and single mesh size gill nets (1.5 × 30 m) of mesh sizes 19.5, 24, 29, 35, 40, and 45 mm were used to

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Table 2. Estimated number of Arctic char in the estimation area for four length-classes in August 1997.

Length-class (mm)	M_1	M_2	C_1	R_1	C_2	R_2	N (±95% CL)
60–89	412	360	413	25	404	46	6695 (5217–8696)
90–119	162	171	188	15	307	30	2885 (2164–3949)
120–149	60	59	62	3	549	26	2301 (1313–3418)
150–179	4	7	7		47	1	273
Total	638	597	670	43	1307	103	

Note: M_1, number of fish marked at the first marking; M_2, number of fish marked at the second marking; C_1, number of fish captured and controlled for marks at the first recapture; C_2, number of fish captured and controlled for marks at the second recapture; R_1, number of recaptured marked fish at the first recapture; R_2, number of recaptured marked fish at the second recapture.

remove fish (cumulative catch) from the population. CPUE was calculated as the number of fish per NORDIC gill net during 12 h of sampling. All gill nets were set randomly throughout the lake.

The total number of fish in the population in the length-classes 60–179 mm was estimated during July to October 1998 (mark–recapture experiment 2). Marking of fish was carried out during July 7 to August 4, 1998, and recapture took place during August 17 to October 18, 1998. Fish were caught with benthic funnel traps placed at depths from 15 to 30 m (Fig. 1) and marked with removal of the pelvic and adipose fins in different combinations. Regeneration of fins on fish marked in 1997 made it possible to distinguish between fish marked in different years. The fish were observed for 30–60 min after marking. Less than 2% of the fish were found not to be in an acceptable state for release and were killed. Recapture was carried out with NORDIC gill nets and single mesh size bottom gill nets (1.5 × 10 and 1.5 × 30 m) of mesh sizes 8, 10, 12.5, and 14.5 mm. The recapture effort was evenly distributed throughout the lake (Fig. 1). The number of fish in each length-class (±95% CI) was estimated according to the adjusted Petersen estimate (Ricker 1975). Relative selectivity for Arctic char >250 mm was calculated as the frequency of this length-class in NORDIC gillnet samples from 1998 divided by its estimated frequency in the catchable population (>60 mm). Due to few catches and no significant decline in CPUE over time, it was not possible to assess the abundance or the gillnet selectivity for length-classes 180–249 mm. However, the expected frequency of these length-classes is small, and bias in the estimate of selectivity for fish >250 mm due to the exclusion of 180- to 249-mm Arctic char should be minor.

Estimation of population structure

An estimate of the age and length distribution for the four length-classes 60–89, 90–119, 120–149, and 150–179 was obtained by correcting the catches from NORDIC gill nets for the relative selectivity obtained through mark–recapture experiment 1. Due to low recapture rates, experiment 2 was not used for this purpose. The estimated number of fish of a given length-class in gillnet catches corrected for relative selectivity was calculated as the number in gillnet catches divided by the relative selectivity of the respective length-class. Age distribution was estimated by means of an age–length key (see Ricker 1975) based on the estimated length distribution of fish from 60 to 179 mm. The proportion of the population in length-classes exceeding 180 mm was small, and bias due to exclusion of these fish should be negligible. Age groups younger than 2+ years were not fully grown into the smallest length-classes caught in the NORDIC gill nets and were therefore omitted.

Statistical analyses

Discrepancy between the estimated distribution of fish in different length-classes in the estimation area (mark–recapture experiment 1) and the distribution of length-classes in NORDIC gillnet catches was tested using Pearson's χ^2 test for goodness-of-fit. The relationship between CPUE and the cumulative number of large Arctic char (>250 mm) caught was analysed using least squares linear regression. The weight–length relationship was described by a power function using nonlinear techniques (SPSS/PC 7.0 computer package). Differences in the slope of the weight–length relationship were tested with Student's t test (two-tailed). Discrepancy between length distributions of NORDIC gillnet samples from different strata of the lake and discrepancy between length distributions of fish marked and recaptured with electric fishing gear in mark–recapture experiment 1 were tested using the Kolmogorov–Smirnov two-sample test (two-tailed). Significance was accepted at the 5% level.

Results

Gillnet selectivity

Selectivity for length-classes 60–179 mm (mark–recapture experiment 1)

Overall, 1235 fish from the different length-classes were marked in the estimation area and 1977 fish were controlled for recapture in 1997 (Table 2). In total, 146 marked fish were recaptured (112 with electric fishing gear and 34 with single mesh size gill nets), giving a frequency of recapture of 0.12. The length distribution of fish marked and released and fish controlled for marks differed, as the subsequent recaptures caught a higher proportion of larger length-classes (Fig. 2A). However, the length distribution of fish marked and released and fish recaptured with electric fishing gear did not differ significantly (Kolmogorov–Smirnov $Z = 0.556$, $P = 0.917$). Ten fish were recaptured more than one subsection away from the subsection in which they were marked. During the experiment, no marked fish were recaptured with gill nets set at depths >3 m or in shallow waters outside the estimation area. The estimated number of fish in the estimation area from length-class 60–89 mm to length-class 150–179 mm declined with increasing fish size (Table 2). However, due to only one recapture, it was not possible to obtain a reliable estimate of the number of fish in the length-class 150–179 mm.

A total of 68 fish in length-classes from 60 to 179 mm were captured with NORDIC gill nets in the estimation area during August 1997 (Table 3). There was a pronounced increase in relative selectivity of the NORDIC gill nets with increasing length of the fish for length-classes 60–179 mm (Table 3). The selectivity increased by a factor of 64 from the length-class 60–89 mm to the length-class 150–179 mm.

Our estimates of the relative selectivity of the NORDIC gill nets with respect to length-classes 60–179 mm were

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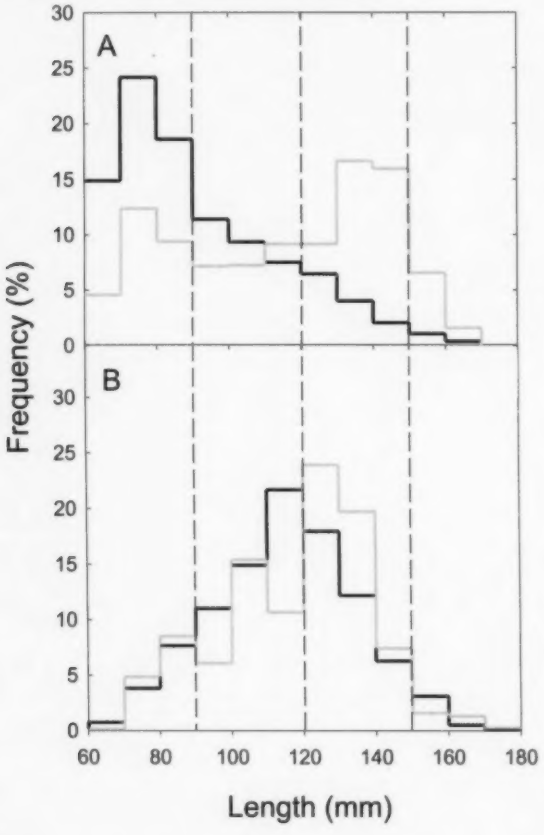
Fig. 2. Length distributions of Arctic char from 60 to 179 mm that were marked and released (black line) and controlled for recaptures (grey line) in (A) mark–recapture experiment 1 and (B) mark–recapture experiment 2. Vertical broken lines indicate the four length-classes estimated separately.



compared with earlier findings for brown trout (*Salmo trutta*) by Jensen and Hesthagen (1996) (Fig. 3).

Selectivity for the length-class >250 mm (successive removal experiment in combination with mark–recapture experiment 2)

In total, 1724 fish in length-classes 60–179 mm were caught in the funnel traps and marked during 1998 in mark–recapture experiment 2 (Table 4). Of the 740 fish controlled for marks, 52 were marked in 1998 (Table 4), giving a frequency of recapture of 0.08. The length distribution of fish controlled for recaptures differed from the length distribution of fish marked and released (Fig. 2B). Except for the estimate for the length-class 120–149 mm, the confidence intervals are broad due to few recaptures. The estimate for the length-class 60–89 mm is not reliable due to only one recapture out of 142 marked fish.

All together, 43 Arctic char (>250 mm) were removed from the population during 1997 and 1998. CPUE for this length-class declined from 1.0 to 0.1 from the first to the last sampling. The relationship between the cumulative number caught in the length-class >250 mm (x) and CPUE (y) was described by the regression line $y = 1.018 - 0.019x$ ($r^2 = 0.65$, $P = 0.008$), which gives an initial abundance of 53 (±42–90) Arctic char >250 mm at the start of the sampling in 1997. This indicates a frequency in the population >60 mm (see Table 4) of 0.0016 for large char (>250 mm). The frequency of Arctic char >250 mm in catches from NORDIC gill nets in 1998 was 0.032 (13 fish), giving a combined relative selectivity for these fishes of 20 compared with the length-classes 60–179 mm. This estimate is conservative, since the number of Arctic char >250 mm in 1998 would be lower than the estimated initial abundance due to the removal of 29 Arctic char >250 mm from the population in 1997.

Size and age structure

The size distributions in catches from NORDIC gill nets were dominated by the length-classes 100–150 mm (Fig. 4). The same number of fish were caught on NORDIC gill nets in both 1997 and 1998. Length distributions were relatively even in both sampling years, although the 1998 sample consisted of slightly smaller fish. The length distribution from NORDIC gillnet catches could be described as unimodal, with a prolonged right-hand tail of large fish. The age distribution from NORDIC gillnet catches was unimodal and was characterised by a modal age varying between 5+ years in 1998 and 7+ years in 1997 (Fig. 5). There was no significant difference between the length distribution of fish (60–179 mm) sampled with NORDIC gill nets in the estimation area and the length distribution of fish (60–179 mm) sampled with NORDIC gill nets in the rest of the lake in 1997 (Kolmogorov–Smirnov $Z = 1.11$, $P = 0.17$). Nor was there a significant difference between the length distribution of fish (60–179 mm) sampled with NORDIC gill nets in shallow areas (<5 m depth) and the length distribution of fish (60–179 mm) sampled with NORDIC gill nets in the rest of the lake in 1998 (Kolmogorov–Smirnov $Z = 1.28$, $P = 0.07$).

The exponent in the power function describing the weight–length relationship (±SE) for length-classes <180 mm was 2.93 (±0.15), 3.02 (±0.12), and 3.47 (±0.03) for fish sampled with NORDIC gill nets in the estimation area in August 1997, fish sampled in the rest of the lake in 1997, and fish sampled in the whole lake in 1998, respectively. This exponent did not differ significantly between fish sampled with NORDIC gill nets in the estimation area in August 1997 and fish in the same length-classes sampled with NORDIC gill nets in the rest of the lake in July and August 1997 ($t = 0.56$, $P = 0.57$) or fish sampled with NORDIC gill nets in 1998 ($t = 0.86$, $P = 0.37$).

The catches from NORDIC gill nets gave pronounced biases in the population size and age structures in relation to the size and age structures derived at through direct estimates of gillnet selectivity. While the frequency of length-classes 60–179 mm showed a distinct modal structure in NORDIC gillnet samples, the estimated frequency declined regularly with increasing size (Fig. 5). The same pattern is apparent for the age distribution (Fig. 5). The estimated frequency of length-classes 60–149 mm in mark–recapture experiment 1 (Table 3) differed significantly from the frequency of the same length-classes in NORDIC gillnet catches when all of the variation within the estimated confidence intervals (Table 2) was adjusted for ($\chi^2 > 54.4$, df = 4, $P < 0.001$).

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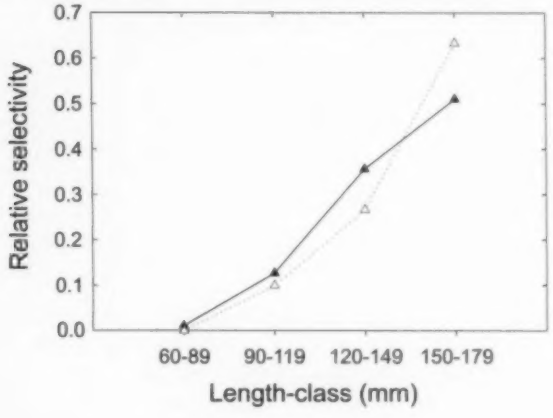
Table 3. Relative selectivity of NORDIC gill nets for four length-classes of Arctic char based on the estimated frequency of each length-class in the length interval 60–179 mm and the observed frequency of the same length-classes in NORDIC gillnet catches from the estimation area in August 1997.

Length-class (mm)	Number in NORDIC gillnet catch	Frequency in NORDIC gillnet catch	Frequency in estimate	Relative selectivity
60–89	3	0.04	0.56	0.07
90–119	18	0.27	0.24	1.13
120–149	41	0.60	0.19	3.16
150–179	6	0.09	0.02	4.50

Fig. 3. Relative selectivity of NORDIC gill nets for four length-classes of Arctic char (solid triangles). The selectivity values obtained for Arctic char in Øvre Skardørsjø are compared with previously published values for brown trout (Jensen and Hesthagen 1996) (open triangles). To obtain comparable values, the sum of the relative catchability for the four length-classes was scaled to 1.



Twelve out of 46 fish >250 mm, four out of 31 fish 180–249 mm, and two out of 794 fish <180 mm were recorded to have fish in the stomach contents.

Discussion

The increase in gillnet selectivity with increasing Arctic char length in the present study is in accordance with previous direct estimates of size selectivity for NORDIC gill nets on brown trout (Jensen and Hesthagen 1996). The gillnet samples gave a fundamentally erroneous size and age distribution. The observed size and age distributions were evidently a direct result of bias in the sampling procedure, as small and young fish were underrepresented in the catches due to increasing catchability with increasing fish size.

Both statistical and methodological uncertainties are involved in estimates obtained by mark–recapture methods (see Ricker 1975), and the assumptions of mark–recapture experiments are seldom met in natural populations (Gatz and Loar 1988). However, the basic assumptions were not significantly violated in the present experiments. Marking fish with fin removal is not found to induce significant increases in morality in short-term studies (Mears and Hatch 1976; O'Grady 1984; Johnsen and Ugedal 1988). In mark–recapture experiment 1, few fish were recaptured more than two subsections away from the subsection in which they were marked, and no fish were recaptured outside the estimation area, indicating negligible migration. The length distributions of marked fish and fish caught and controlled for recapture were heterogeneous in both mark–recapture experiments. This was not induced by size-biased mortality, as length distributions were consistent between marked and recaptured fish caught with electric fishing gear. Recruitment into length-classes was also negligible due to slow growth of the fish and the short duration of experiments. The heterogeneous length distributions therefore indicate differences in size selectivity between sampling gears used in catching fish for marking and for recapture. However, Ricker (1975) showed that unequal vulnerability to sampling gear for different fish sizes did not introduce major errors into mark–recapture estimates. Estimating the abundance of each length-class separately reduces this bias further. Hence, the pronounced and highly significant difference between the estimated frequency of each length-class in mark–recapture experiment 1 and the estimated frequency of each length-class in the NORDIC gillnet catches cannot be explained by biases in the mark–recapture experiment.

Table 4. Estimated number of Arctic char in the total population for four length-classes in July–October 1998 (mark–recapture experiment 2).

Length-class (mm)	*M*	*C*	*R*	*N* (±95% CL)
60–89	142	84	1	6078
90–119	678	211	7	17 335 (9347 – 37 881)
120–149	795	398	39	7960 (5864 – 11 094)
150–179	109	47	5	880 (416–2031)
Total				32 253

Note: *M*, number of fish marked; *C*, number of fish captured and controlled for marks; *R*, number of recaptured fish.

The age–length key assumption of equal catchability for all fish within a length-class (see Ricker 1975) was probably violated, since catchability normally increases with size for both electric fishing gear (Bohlin et al. 1989) and gillnet series (Jensen and Hesthagen 1996). This implies that our results are conservative with regard to the true age-related bias in gillnet catches, since increased selectivity with increasing length results in an underestimation of smaller, and hence younger, fish.

Deriving whole-lake population structure from supralittoral estimates of gillnet selectivity assumes constant gillnet selectivity throughout the different strata of the lake. The consistency in the weight–length relationship between NORDIC gillnet samples from the estimation area and those from the rest of the lake in 1997 and in 1998 suggests that the retention probability relative to fish length did not differ between strata or between years. Also, the homogeneity of length distributions of NORDIC gillnet samples from the estimation area and those from the rest of the lake, and the similarity of length distributions between years, support a constant size selectivity between different sampling periods and different strata.

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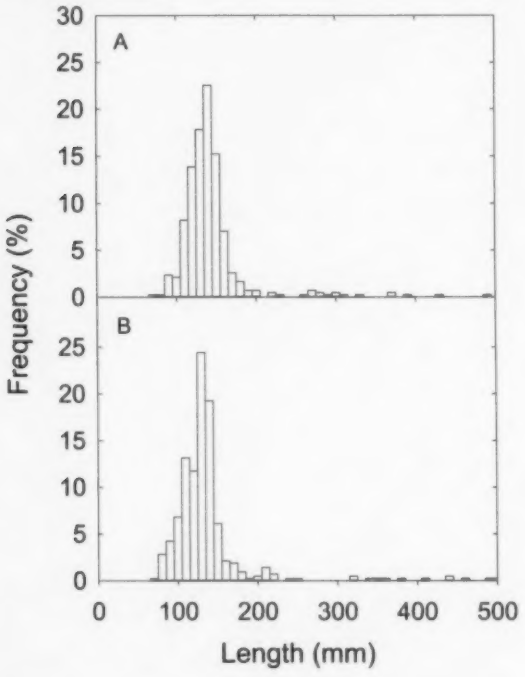
Fig. 4. Length distribution of Arctic char sampled with NORDIC gill nets in Øvre Skarddørsjø during (A) 1997 (N = 426) and (B) 1998 (N = 426).



Removal methods assume constant catchability and no emigration, no natural or unaccounted mortality, and no recruitment of fish into the length-classes being estimated while the successive catches are being made. Although these assumptions to a degree are likely to have been violated in the present study, different violations would have affected the estimate in opposite directions. Further, there was an order of magnitude decline in the CPUE of large Arctic char (>250 mm) over the sampling period. Thus, we conclude that the controlled removal of large Arctic char resulted in the pronounced decline in CPUE.

The process of gillnet capture of fish can be broken down into several sequential events. The probability of a fish being caught by a gill net is equal to the product of the conditional probabilities associated with each event in the process:

$$P(\mathrm{C}) = P(\mathrm{O}) \times P(\mathrm{E}) \times P(\mathrm{R})$$

where P(C) is the probability of a fish being caught by the net, P(O) is the probability of spatial and temporal overlap between net location and fish habitat, P(E) is the probability of the fish encountering the net, and P(R) is the probability of the net retaining the fish. Size selectivity of a gill net may be affected by any of the conditional probabilities. Gill nets are passive fishing gears and depend on the fish actively encountering the net. This requires an overlap between net location and the range of the fish (P(O)), and in order to obtain representative samples from a fish population, P(O) must be equal between length-classes. Given spatial and temporal overlap, Rudstam et al. (1984) emphasised that P(E) is directly proportional to the swimming distance of the fish during the sampling period, given random swim. Probably more often than not, larger fish swim longer distances than smaller fish, thus increasing P(E). The P(E) encompasses the probability of the fish avoiding the net after having sensed it. Smaller fish are more finely tuned in manoeuvring (Breck and Gitter 1983) and may avoid nets proportionately more often. Both P(O) and P(E) are related to fish behaviour and probably often depend on size in a fashion that increases gillnet selectivity with increasing size. Equally, P(R) should increase with increasing mesh size, as nets of smaller mesh sizes are less tensile and flexible than nets of larger mesh sizes. Also, larger fish may be expected to encounter the net with higher speed and, combined with a larger mass, increased energy for entanglement.

Indirect estimates of selectivity for NORDIC multimesh gill nets following Gulland and Harding (1961) demonstrate a relatively constant pooled selectivity for length-classes 5–30 cm (Appelberg et al. 1995). This contrasts with the direct estimates presented in this study and those of Jensen and Hesthagen (1996). The discrepancy between the indirect and direct estimates is probably related to the assumption of equal efficiency for different mesh sizes in the indirect estimates. It was not possible to make interpretations on the selectivity curves for different mesh sizes in the present study. However, an increase in efficiency with increasing mesh size for length-classes 60–179 mm may be expected to comply with that found by Jensen and Hesthagen (1996). This increase is exponential and more pronounced than found earlier for salmonoids by Hamley (1980) and Jensen (1995*a*). Variability of the functional relationship between fish size and gillnet efficiency is common (Hamley 1980), suggesting size-related differences in behaviour. Fish behaviour depends not only on size but probably also on the frequency of different-sized fish in the surroundings.

Experimental studies demonstrate that alternation of predation hazards may alter behaviour and habitat selection (e.g., Milinski 1993). Habitat shift in response to predation risk by conspecifics has been demonstrated in Arctic char (Damsgård and Ugedal 1997). Accordingly, the catchability of the smallest length-classes in the present study is suspected to be affected by cryptic antipredator behaviour. The presence of cannibals may affect swimming distance negatively or induce epibenthic hiding behaviour and hence decrease P(E) and P(O).

Arctic char is a phenotypically variable species, and bimodal size distributions are often associated with habitat heterogeneity and polymorphism (Griffiths 1994). Bimodal size distributions may arise from differences in growth rate within cohorts and accumulation of fish in the modal length-classes due to stagnation in growth (Griffiths 1994). Occasionally, stagnation in growth is observed in upper-mode fish from lacustrine Arctic char populations with the observed bimodal size distribution (Gullestad and Klementsen 1997), but it is usually not apparent (e.g., Klemetsen et al. 1985; Sparholt 1985; Hammar 2000). In addition, bimodal age distributions have been reported (Svenning and Borgstrøm 1995; Hammar 2000). We suggest that the observed bimodal size (and age) distributions from these populations may arise from gillnet selectivity (Fig. 6). The effect of an exponential increase in catch efficiency with increasing length on the NORDIC gillnet combination of mesh sizes is an observed

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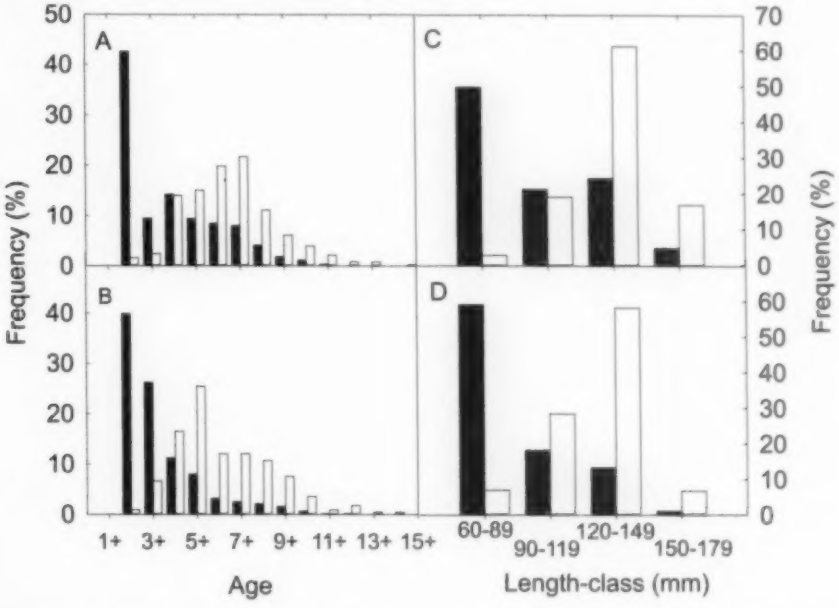
Fig. 5. Observed (open bars) and estimated (solid bars) frequencies of age-classes from 2+ to 15+ years and length-classes 60–179 mm of Arctic char sampled with NORDIC gill nets in Øvre Skarddørsjø in (A and C) 1997 and (B and D) 1998. The number of fish in NORDIC gillnet samples was 397 in Figs. 5C and 5D, and number of fish aged (with number of fish aged in NORDIC gillnet samples in parentheses) was 511 (378) in Fig. 5A and 253 (225) in Fig. 5B.



unimodal population size structure (Fig. 6B). Introducing an abrupt increase in catch efficiency at some size gives rise to an observed bimodal distribution (Fig. 6C). The abrupt increase in the pooled relative efficiency for length-classes >20 cm in Fig. 6C is intended to model a behavioural change associated with a niche shift to piscivory. The relative selectivity for Arctic char >250 mm in Øvre Skarddørsjø was estimated to be approximately five times larger than the relative selectivity for fish in the length-class 150–179 mm. This increase in selectivity may be related to an ontogenetic size-dependent diet shift where the fish switch to an active predacious feeding strategy that abruptly increases $P(E)$. With respect to the estimated low abundance of large Arctic char in Øvre Skarddørsjø, only a hint of bimodality in the gillnet samples from this lake is not surprising.

The complex ontogenetic growth and mortality pattern suggested by Johnson (1976) is frequently associated with observed uni- or bi-modal size distributions and bell-shaped age distributions in populations of Arctic char (Sparholt 1985; Parker and Johnson 1991; Gullestad and Klemetsen 1997). The observed population structure from NORDIC gillnet samples in Øvre Skarddørsjø with a unimodal size distribution and a bell-shaped age distribution is also in accordance with Johnson's (1976) hypothesis. However, the estimated population structure based on gillnet catches corrected for size selectivity is fundamentally different. The estimated frequency of each age-class decreases with increasing age. Likewise, the estimated frequency of each length-class between 60 and 179 mm decreases with increasing size. The difference between the observed and estimated population structure in Øvre Skarddørsjø was a direct result of a pronounced increase in selectivity for the NORDIC gillnet series with increasing fish size and, accordingly, age. Although errors in ageing of fish cannot be ruled out, erroneous age determination is not required to explain the unimodal age distribution in our gillnet catches.

A complex ontogenetic growth and mortality pattern is not essential to explain the observed population structure of Arctic char in Øvre Skarddørsjø, as the population may simply be structured by a high mortality, especially in the small and young fish coupled with slow growth. The right-hand tail of the observed size distribution consists of rapidly growing cannibals. This does not, however, exclude a top-down regulation of recruitment, as proposed by Johnson (1976).

Our study demonstrates that the use of multimesh gill nets for assessing population structure may result in profound biases with regard to interpretations of size and age structure, and hence, biological interactions, if the sampling bias is not accounted for. Further, we suggest that gillnet selectivity will be an innate property of populations depending on their size structure, which points to an intrinsic limitation of gill nets as sampling gear for estimating population structures. Present methods for direct estimates of population structures through, for example, mark–recapture experiments are tedious. However, indirect methods for estimating gillnet selectivity, which rely on comparing size distributions of fish caught by different mesh sizes, are deficient because of the assumptions of equal efficiency of the different mesh sizes. Future research on gillnet selectivity should to a larger extent differentiate between events in the capture process and take into account fish behaviour and its dependence on external factors. The observed variation in heights of selectiv-

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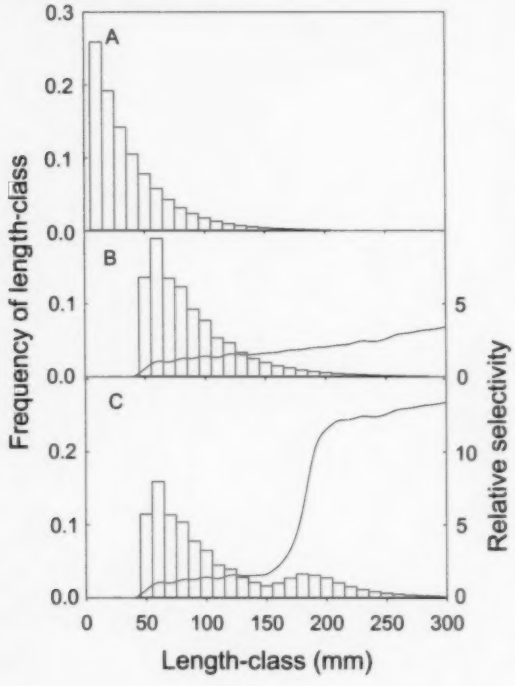
Fig. 6. (A) Hypothetical population frequency distribution of sizes and (B) the resultant distribution of a hypothetical size-selective gillnet sample when the pooled relative selectivity for a combination of mesh sizes equalling the NORDIC gill net is calculated according to Jensen (1995*b*), excluding a factor for twine thickness. The exponent for increase in the height of the selectivity curve was obtained from Jensen (1995*a*). The observed frequency of each length-class in the sample is the product of the frequency of the length-class in the initial distribution (Fig. 6A) and the pooled relative selectivity for a given length-class as indicated by the line in Fig. 6B. (C) Observed size distribution resulting from modifying Fig. 6B by adding an abrupt increase in the pooled relative efficiency.



ity curves also implies that care should be taken in direct comparisons of size and age distributions from gillnet catches from different populations. However, time series of samples obtained with standardised multimesh gill nets may give valuable information about relative changes in population structure, which is often a main issue in monitoring programs.

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Growth variation and water mass associations of larval silver hake (*Merluccius bilinearis*) on the Scotian Shelf[1]

Jennifer A. Jeffrey and Christopher T. Taggart

Abstract: Otolith microstructure is shown to be ideal for assessing age and growth in silver hake (*Merluccius bilinearis*) larvae and is used to examine growth among individuals and cohorts. Larvae collected from Western Bank, Scotian Shelf, in September, October, and November 1997 defined three monthly cohorts identified using inferred hatch dates. Total length-at-age relations did not differ between the September and October cohorts despite substantial differences in growing degree-day (435 versus 318°C·d) and zooplankton (potential prey index) wet biomass (0.15 versus 0.27 $g \cdot m^{-3}$). Larvae collected off-bank in September exhibited a growth advantage of >0.10 $mm \cdot d^{-1}$ relative to larvae collected on-bank. Greater variability in growth rate within cohorts and among water masses implies that cohort-averaged growth and survival (based on growth) estimates can be biased by overrepresentation of a single water mass. The focus on growth variability, and its relationship to survival, should be on individuals within cohorts and not on cohort-averaged estimates. We hypothesize that growth, and perhaps survival, in silver hake larvae on the Scotian Shelf is most easily explained by variation in physical oceanographic processes.

Résumé : Nous montrons que la microstructure des otolithes est un outil idéal pour évaluer l'âge et la croissance chez les larves de merlu argenté (*Merluccius bilinearis*), et nous nous en servons pour examiner la croissance parmi les individus et les cohortes. Les larves prélevées sur le banc Occidental, sur la plate-forme Néo-Écossaise, en septembre, octobre et novembre 1997, représentaient trois cohortes mensuelles identifiées par leurs dates d'éclosion supposées. Les relations de la longueur totale selon l'âge ne différaient pas entre les cohortes de septembre et d'octobre malgré des différences substantielles dans les degrés-jour de croissance (435 c. 318°C·jour) et la biomasse humide de zooplancton (indice des proies potentielles) (0,15 c. 0,27 $g \cdot m^{-3}$). Les larves recueillies à l'extérieur du banc en septembre présentaient un avantage de croissance de >0,10 $mm \cdot jour^{-1}$ par rapport aux larves récoltées sur le banc. La plus grande variabilité du taux de croissance à l'intérieur des cohortes et entre les masses d'eau implique que les estimations de la moyenne par cohorte de la croissance et de la survie (d'après la croissance) peuvent être biaisées par la surreprésentation d'une masse d'eau. L'examen de la variabilité de la croissance, et de sa relation avec la survie, devrait porter sur les individus au sein des cohortes et pas sur les estimations des moyennes par cohorte. Nous posons que, pour les larves de merlu argenté de la plate-forme Néo-Écossaise, ce sont des variations dans les processus océanographiques physiques qui expliquent le mieux la croissance, et peut-être la survie.

[Traduit par la Rédaction]

Introduction

A principal goal of fisheries oceanography is the testing of hypotheses proposed to explain variations in the year-class strength (recruitment) of marine fishes. It was originally hypothesized that year-class strength was determined during the early life history, usually within the first year of life (Hjort 1914, 1926). Several variations on the original hypothesis have evolved (e.g., Shepherd and Cushing 1980; Iles and Sinclair 1982). Year-class strength is some function of larval survival that may be a function of growth (Houde 1987). Variation in growth (hence survival) during the prerecruitment period is thought to contribute to recruitment variation through regulation of the length of the larval stage (Houde 1987; Pepin 1991). Larger or faster growing larvae may experience a contracted larval period, reducing the time that they are subject to predation and reducing the probability of starvation associated with small size and limited motility (e.g., Miller et al. 1988; Rice et al. 1993; Meekan and Fortier 1996).

Variability in growth rate is a function of biological conditions (e.g., feeding success: Govoni et al. 1985) and oceanographic conditions (e.g., temperature and turbulence: Pepin 1991; Gallego et al. 1996). The ability to predict growth rate using these kinds of variables and their associated parameters may lead to survival and ultimately recruitment predictions from the larval stage.

Several studies have examined growth rates (inferred from size-at-age relations) that are averaged over populations or cohorts (see Rice et al. 1987). However, averaging growth rate estimates may mask relations between growth rate and environmental conditions (Rutherford and Houde 1995). Further, as size-selective mortality (starvation or predation

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J.A. Jeffrey and C.T. Taggart.[2] Department of Oceanography, Dalhousie University, Halifax, NS B3H 4J1, Canada.

[1]This paper is a GLOBEC Canada contribution.
[2]Author to whom all correspondence should be addressed.
e-mail: chris.taggart@dal.ca

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related or both) acts on individuals, focussing on population- or cohort-averaged growth rates may preclude testable predictions of survivorship when growth rate is used as the predictor (Pepin 1989). Thus, the analysis of growth rate variation among individuals within well-defined cohorts may be key to resolving the processes responsible for growth rate variation and ultimately survival in larval fish (e.g., Chambers and Miller 1995; Meekan and Fortier 1996).

Growth rate variation in larval fish can be resolved using otolith microstructure (Campana and Neilson 1985). The age of an individual can be determined and individual growth rates can be calculated using somatic or otolith size-at-capture. Perhaps of greater utility is the variation in the width of daily otolith increments that may be related to daily variation in somatic growth rate and therefore provides a time series of growth variation over the life of individual larva (see review by Campana and Neilson 1985).

It is not clear that somatic and otolith growth are correlated on a day-to-day basis. Some researchers report a decoupling of the two when growth is variable (i.e., fast-growing larvae have smaller otoliths relative to body size than slow-growing larvae: Mosegaard et al. 1988; Secor et al. 1989). Others report that larval size and otolith size remain highly correlated regardless of growth rate (Dickey et al. 1997), and it is generally accepted that otolith growth is a "running average" of somatic growth (Campana and Neilson 1985). Despite uncertainties in the accuracy of back-calculation of larval size or growth rate from otolith size, questions concerning relative growth differences among larvae can be addressed given the assumption that larger otolith size-at-age corresponds to greater somatic size-at-age (Hare and Cowen 1995). For the questions that we address below, only relative comparisons of growth are important. Therefore, the precise nature of the otolith–fish size relation and the accuracy of the back-calculated larval size from otolith size is not critical.

Silver hake (*Merluccius bilinearis*) is a model species for testing growth and environment hypotheses in larvae at a variety of temporal and spatial scales, and Western Bank (Scotian Shelf) is an ideal location to conduct such tests. Mature silver hake are benthopelagic and spawn on the Scotian Shelf in summer and autumn (Scott and Scott 1988). The larvae are pelagic for 3–5 months posthatch and are generally found in the upper mixed layer (Fortier and Villeneuve 1996). There are few studies of somatic or otolith growth in silver hake larvae (>20 mm only: Nichy 1969; juveniles: Koeller et al. 1989; larvae: Buckley et al. 1993). However, Pannella (1971), in his pioneering work on otolith microstructure, concluded that silver hake deposit daily otolith increments after he estimated an average of 360 increments between the annuli of 3- and 4-year-old silver hake. Further, daily increments are confirmed for several close relatives: *Merluccius productus*, *M. angustimanus*, *M. capensis*, and *M. paradoxus* (Brothers et al. 1976; Bailey 1982; Morales-Nin 1987). Finally, Buckley et al. (1993) observed a weak inverse relation between mortality and growth in silver hake larvae in the laboratory. Thus, growth variability in the field may, in part, explain survival variability. To our knowledge, otolith microstructure has not been used to examine growth rate in larval silver hake in the field. This is surprising, as (apart from the benefits of acquiring new knowledge) protracted spawning and the persistence of eggs and larvae in regions like Western Bank over much of the autumn (O'Boyle et al. 1984; Reiss et al. 2000) provide an ideal opportunity to examine age and growth among larvae that have experienced different oceanographic conditions in time and space.

In this paper, we provide evidence that otolith microstructure is appropriate for assessing age and growth rate variation in silver hake larvae. Subsequently, we use the microstructure to test the working hypothesis that a significant proportion of growth variation among monthly cohorts and among individuals within cohorts is most simply explained by temporal and spatial variation in oceanographic conditions.

Materials and methods

Oceanographic sampling

Larvae and hydrographic data were collected in the Western Bank region (Fig. 1) between 15 and 26 September, 25 October and 10 November, and 18 November and 5 December 1997. Three monthly cohorts and two subcohorts were defined using larvae collected during the three periods. Monthly cohorts are generally defined by month of collection and specifically defined as a representative sample of larvae aged at ≤25 d at capture, with nonoverlapping hatch dates, collected in each month (September, October, and November cohorts, respectively; Table 1). Two subcohorts are defined as larvae aged between 10 and 20 d at capture and collected in each of September and October (referred to as subcohorts 1 and 2, respectively).

Larvae were collected in September and November using a 63-cm-diameter BONGO (Posgay and Marak 1980) fitted with 333-μm-mesh nets. The gear was towed in a double-oblique manner to within 5–10 m of the bottom (November) or to ~65% of the bottom depth (September). Plankton collected from one net was preserved in 5% (v/v) buffered formalin–seawater and the other in 95% (v/v) ethanol–freshwater. Larvae were collected in different strata in October using a 0.25-m^2-opening BIONESS (Sameoto et al. 1980) fitted with ten 243-μm-mesh nets. Two sets of samples were collected at each station, each set consisting of four collections from different strata and one depth-integrated collection from ~10 m above bottom to the surface (i.e., 10 nets per station, five per set). One set of collections was preserved in ethanol and the other in formalin as above. Larvae used for otolith analysis were from the samples preserved in ethanol. Volume filtered was measured for all nets using calibrated flowmeters mounted off-centre in the gear opening.

Zooplankton wet biomass ($g \cdot m^{-3}$) was used as an index of the potential prey field of the larvae and was measured using the formalin-preserved BONGO collections (September and November) and the station average of the four formalin-preserved depth-discrete BIONESS collections (October). Wet biomass was measured after fish larvae and eggs, ctenophores, and rare large plankters (amphipods, euphausiids, etc., nominally >10 mm in length) were removed to help reduce biasing the index of the potential prey field. The remaining plankton was drained on a small-mesh screen (<243 or <333 μm as required) until no longer dripping and weighed. Biomass estimates (station specific or station averaged as required) were calculated to match the various larval collections at their different scales of analyses.

Conductivity and temperature at depth were measured at each sampling station using a Seabird® Electronics SBE 25e CTD and salinity was derived and density was calculated from temperature, salinity and depth using the equation of state. Temperature time series (0.5 h resolution) were collected between 1 August and 6 December 1997 at two moorings separated by ~15 km on the crest of

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Fig. 1. Bathymetric charts of Western Bank, Scotian Shelf, showing the isobaths and locations where silver hake larvae were collected in 1997 in (*a*) September, (*b*) October, and (*c*) November and (*d*) location of current meter moorings where temperature time series were collected. Solid symbols in Figs. 1*a*–1*c* represent locations included in the monthly cohorts, and open symbols represent locations where other larvae not used in otolith analyses were collected. The plus sign in Fig. 1*a* shows the location where additional larvae included in the subcohort 1 analyses were collected.

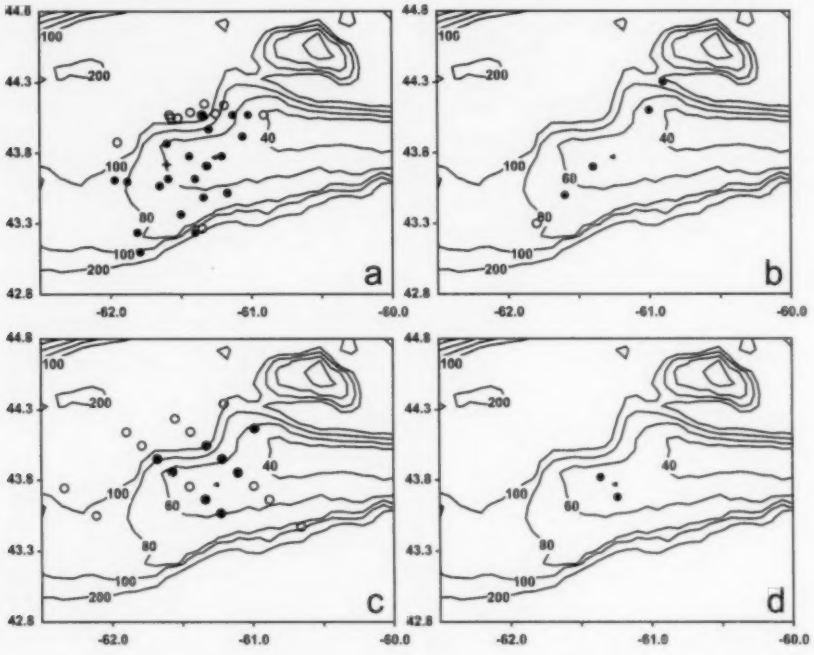

Table 1. Summary of Western Bank silver hake larvae collected, analyzed, and assigned to each monthly cohort.

Cruise	Sampling period	Estimated number of larvae available	Number of aged larvae	Number of larvae aged ≤25 d	Cohort	Inferred cohort hatch dates
Q-242	15–26 Sept.	9526 (33)	175 (23)	165 (21)	September	28 Aug. to 20 Sept.
H97-063	25 Oct. to 10 Nov.	135 (5)	128 (5)	101 (4)	October	3–25 Oct.
N97-070	18 Nov. to 5 Dec.	205 (19)	52 (10)	17 (8)	November	29 Oct. to 17 Nov.

Note: Values in parentheses represent the number of stations from which larvae were collected.

Western Bank (Fig. 1*d*). Each mooring consisted of an Interocean S4 current meter (with temperature probe) moored at 11 m depth and a temperature recorder (Vemco Inc., Dartmouth, N.S.) moored at 2 m depth. Series data were smoothed using a 25-point moving median and daily temperature estimates were calculated as the average of the daily noon (UTC) values of the four series. Growing degree-day (GDD) estimates were calculated as the running sum of the average daily temperature estimates (°C) calculated for a 25 d period beginning with the earliest inferred hatch date for each monthly cohort.

Larvae and otolith microstructure

All formalin-preserved fish larvae collected with the BONGO were removed, identified to species, and enumerated. The abundance of formalin-preserved silver hake was used to select stations and the number of ethanol-preserved larvae destined for otolith analysis. Ages (d) were obtained for 175 of the 9526 silver hake collected in September that represented 23 of the 33 stations where larvae were collected (Table 1). Stations with <25 larvae were not included in the among monthly cohort growth analyses. In November, all ethanol-preserved larvae ($N = 205$) were removed from collections ($N = 19$; Fig. 1*c*) that had larvae in the formalin-preserved sample. Poor preservation limited aging to 52 larvae collected from 10 of the 19 stations. Ethanol-preserved fish larvae collected with the BIONESS were treated as above and all silver hake from the four depth-discrete samples per station were used for otolith analysis ($N = 135$). Ages were obtained for 128 larvae collected in October (Table 1).

Total length (TL, ±0.1 mm) of silver hake larvae selected for otolith analysis was measured using a digital image (Sony XC-711 CCD camera and a Wild M5 dissecting microscope) and OPTIMAS® (version 5.2; Optimas Corporation 1995). TL estimates were not corrected for shrinkage in ethanol, as it is dependent on initial length and is highly variable (Fowler and Smith 1983; Jeffrey 2000). Left and right lapilli and sagittae were removed from larvae using insect needles and attached to microscope slides using cyanoacrylate glue or clear nail polish. Age was determined by counting the number of daily otolith increments at 200–1000× magnification (Wild M20) and total otolith area (OA, ±100 μm^2) was measured on a digital image taken at 40–400× magnification (Sony XC-711 CCD camera). Surface areas of individual incre-

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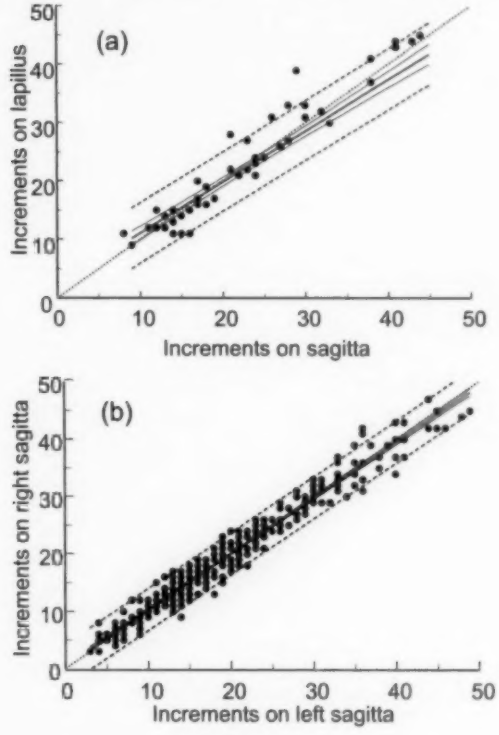
Fig. 2. Scattergrams and least squares regressions of the number of increments (estimated age, d) for (*a*) lapilli and sagittae (lap = 2.3 + 0.88sag; $r^2 = 0.93$, $N = 47$) and (*b*) right and left sagittae (R = 0.56 + 0.97L; $r^2 = 0.96$, $N = 382$) for silver hake larvae collected from September to November 1997 on Western Bank. Solid lines show the regressions and 95% confidence bands. Dashed lines show the 95% confidence bands for the prediction for an individual. The dotted line is the 1:1 relationship.



ments were measured using a Kodak Megaplus 1.4i high-resolution digital image and SigmaScan (version 5.0.0; SPSS Inc. 1999).

There was no difference between the estimated age of randomly chosen sagittae and lapilli within individuals (Wilcoxon sign test, $p = 0.44$, $N = 47$; Fig. 2*a*). Sagittae were easier to remove and read than lapilli and are used throughout. Sagittae are considered ideal because (*i*) Pannella (1971) validated daily increment deposition on the sagittae of silver hake, (*ii*) we found that age estimates using the left and right sagittae within individuals were the same (Wilcoxon sign test, $p = 0.54$, $N = 382$; Fig. 2*b*), (*iii*) repeated blind age estimates for sagittae by the principal reader were not different (Wilcoxon sign test, $p = 0.096$, $N = 50$), (*iv*) the surface area estimates for left and right sagittae were the same (Wilcoxon sign test; $p = 0.89$, $N = 311$), and (*v*) no relation was observed between the difference in the age and the difference in surface area between sagittal pairs within individuals, suggesting that resolution of otolith increments related to otolith size is not likely a confounding factor in aging (Campana et al. 1987). Ages estimated using a subset of sagittae by the principal author and an independent experienced reader were significantly different (Wilcoxon sign test, $p < 0.01$, $N = 12$), although the maximum difference was 2 d. The actual age of the larvae used for interreader comparisons is unknown, and therefore, accuracy of aging cannot be assessed. As all ages were determined by the principal author, we assume that any bias is constant and we are confident that estimates from the sagittae accurately reflect the relative ages. Absolute ages and dates of initial increment formation remain uncertain, although we do not consider it critical for the focus on growth rate variation given the assumption that the time of initial increment deposition is constant for all larvae (Jenkins and Davis 1990). We assume that increment deposition begins at hatch for the estimation of relative ages. The average age and average surface area are reported for age and otolith size estimates when both sagittae were available. When only one was available, the single estimate was used.

Data analyses

The relationship between sagittal OA and larval TL was determined separately for larvae collected each month and the slopes were compared using analysis of covariance (ANCOVA). TL-at-age relations were determined for monthly cohorts (larvae ≤25 d old) when a sufficient range of ages and sizes were available and the slopes were compared using ANCOVA. Residuals of the TL-at-age relations for the September and October cohorts were examined in relation to geographic location of capture and water mass characteristics at the time of capture. Additional larvae collected from other water masses in September were examined and average somatic growth rates (ASGR, $mm \cdot d^{-1}$) and average otolith growth rates (AOGR, $\mu m \cdot d^{-1}$) were calculated for individuals aged between 10 and 20 d at capture in September and October to examine growth variation within subcohorts:

$$(1) \qquad ASGR = \frac{TL_c - TL_h}{age}$$

$$(2) \qquad AOGR = \frac{OA_c - OA_h}{age}$$

where TL_c is TL at capture and TL_h is TL at hatch as determined from the intercept of the monthly cohort-specific TL-at-age relation, and OA_c is OA at capture and OA_h is OA at hatch as estimated by substituting TL_h into the otolith–fish size relation for each monthly cohort. Thus, TL_h and OA_h varied among cohorts and were constant within cohorts. The calculation of ASGR and AOGR assumes that growth rate is constant (linear) for the period examined and that a constant somatic and otolith size-at-hatch represents hatch size for all individuals collected in the same month (cf. Geffen 1995). ASGR and AOGR were each examined in relation to a suite of physical (temperature, salinity, density, GDD, bottom depth, and depth of the surface mixed layer) and biological (zooplankton biomass, total larval concentration, larval silver hake concentration, age and hatch date) variables using bivariate and multiple least squares regression.

Otolith microstructure of 22 individuals collected from on- and off-bank water masses within subcohort 1 (September) was used to examine spatial growth variation among individuals. For these analyses the sagittal surface area ($\pm 100\,\mu m^2$) was measured at each well-defined increment (including the first prominent increment presumed to be the hatch check) when possible. TL-at-age and growth rate-at-age were back-calculated using the biological intercept method (Campana 1990) where the intercept was 1.5 mm TL and 225 μm^2 saggital surface area as recorded for the smallest larva (Fig. 3*a*). Complete TL and growth rate series were obtained for increments 3–10 and increments 4–10, respectively, for five larvae from each water mass. These series were compared among individuals from the two water masses using repeated measures multivariate analysis of variance (rmMANOVA: Chambers and Miller 1995). As the series lengths and sample sizes for the rmMANOVA were limited, the water mass specific TL-at-age and growth rate-at-age series were calculated using all 22 individuals and the slopes (using $\log_e$-transformed data) were compared

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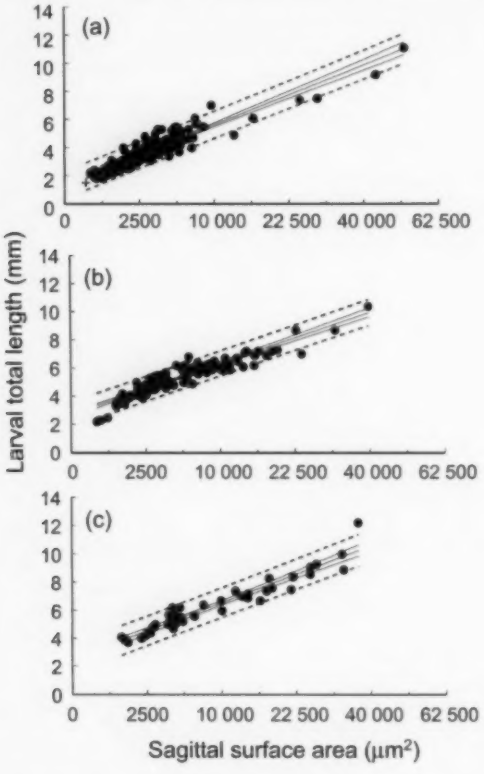
Fig. 3. Scattergrams and least squares regressions of larval TL and sagittal surface area for silver hake larvae collected in 1997 on Western Bank in (*a*) September ($TL = 1.3 + 0.043(OA)^{0.5}$; $r^2 = 0.87$, $N = 168$), (*b*) October ($TL = 2.7 + 0.036(OA)^{0.5}$; $r^2 = 0.88$, $N = 127$), and (*c*) November ($TL = 2.5 + 0.040(OA)^{0.5}$; $r^2 = 0.93$, $N = 44$). Sagittal surface area is square root transformed for the regression. The slope parameters are different among months (ANCOVA, $p = 0.002$). The biological intercept (Campana 1990) used for back-calculations of somatic size from otolith size is indicated by the plus sign in Fig. 3*a*. Solid lines show the regressions and 95% confidence bands. Dashed lines show the 95% confidence bands for the predictions for an individual.



(ANCOVA) between the on- and off-bank larvae. Age of divergence in average growth trajectories for on- and off-bank larvae was estimated by analyses of the back-calculated TL at hatch and TL and growth rate at each age using ANOVA (α set at 0.005 for reduced df in multiple comparisons).

All statistical analyses were performed using Systat (version 8.0; SPSS Inc. 1998) and Number Cruncher Statistical Software (Hintze 1998).

Results

Significant linear relations between TL and OA (square root transformed) were generally observed for the three monthly cohorts (Fig. 3), indicating that otolith size reflects somatic size in silver hake larvae and that OA can be used to back-calculate TL. The slopes of the relations between TL and OA were different (ANCOVA, $p = 0.002$) among monthly cohorts (September, 0.043; October, 0.036; November, 0.040), indicating that relations should be calculated for each cohort. All relations were monotonic with the possible exception of the October cohort where a slight decrease in slope for larvae ≥6 mm TL was apparent (Fig. 3*b*). However, fitting Gompertz, logistic, or exponential functions did not result in any improvement. For the analyses that we rely on, the OA estimates for October were further transformed to linearize the relation $TL = -5.5 + 6.0 \log_{10}(OA^{0.5})$; $r^2 = 0.90$).

Growth variation among monthly cohorts

The representative sample of larvae collected in September had inferred ages that ranged from 3 to 39 d, in October from 3 to 39 d, and in November from 10 to 47 d. Because ages were obtained for only 52 individuals in November, the larvae included in the analyses do not represent all stations in constant proportion. Larvae with ages ≤25 d were selected from each "month" to specifically define the three monthly cohorts as those with inferred hatch dates between 28 August and 20 September (September cohort, $N = 165$), 3 and 25 October (October cohort, $N = 101$), and 29 October and 17 November (November cohort, $N = 17$). The TL-at-age data for the November cohort are presented for comparison with the other cohorts, although the data are not used in further analyses because the sample size is small and larval size and age ranges are limited (Fig. 4). TL-at-age for the September and October cohorts were best fit with linear least squares regression (Fig. 4) and their slopes were not different (ANCOVA, $p = 0.63$).

All larvae in the October cohort and 52% of the larvae in the September cohort were collected during daylight (10:00–22:00 UTC for September and 10:30–21:00 UTC for October). The slopes of the TL-at-age relations for larvae caught during the day ($TL = 1.7 + 0.17age$; $r^2 = 0.73$, $N = 86$) and at night ($TL = 1.4 + 0.19age$; $r^2 = 0.58$, $N = 79$) in September were not different (ANCOVA, $p = 0.35$). Therefore, time of collection is ignored in the TL-at-age analyses.

The temperature time series were similar among locations and depths over the crest of Western Bank (Fig. 5). The GDD estimates were 435°C·d for the September cohort and 318°C·d for the October cohort and represent average daily temperatures of ~17 and ~13°C for larvae in each monthly cohort, respectively. The average zooplankton wet biomass for the stations included in the monthly cohort TL-at-age analyses (i.e., larvae aged ≤25 d) was 0.15 and 0.27 $g \cdot m^{-3}$ for September and October, respectively.

Growth variation within subcohorts

Subcohort 1 (September)

Residuals of the TL-at-age relation for the September cohort revealed a pattern among stations attributable to geographic location. For example, 79% of the larvae collected at one on-bank station had negative residuals, while >90% of the larvae collected at two off-bank stations had positive residuals. Additional larvae were examined from these and other stations to further analyze growth within subcohort 1. ASGR ($N = 164$) and AOGR ($N = 149$) were calculated for larvae aged between 10 and 20 d to prevent comparing growth rates of larvae with widely varying ages. As otoliths do not shrink with preservation, we assume that AOGR provides a better measure of growth throughout life. However,

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Fig. 4. Scattergrams and least squares regressions of larval TL and age for silver hake in September and October cohorts on Western Bank. The slopes of the relations for September (solid circles; TL = 1.6 + 0.18age; r^2 = 0.65, N = 165) and October (open circles; TL = 1.7 + 0.17age; r^2 = 0.82, N = 101) are not significantly different (ANCOVA, p = 0.63). The November cohort (open triangles) is shown for visual comparison only. The solid lines show the regressions and the dotted (September) and dashed (October) lines show the 95% confidence bands for the predictions for an individual.

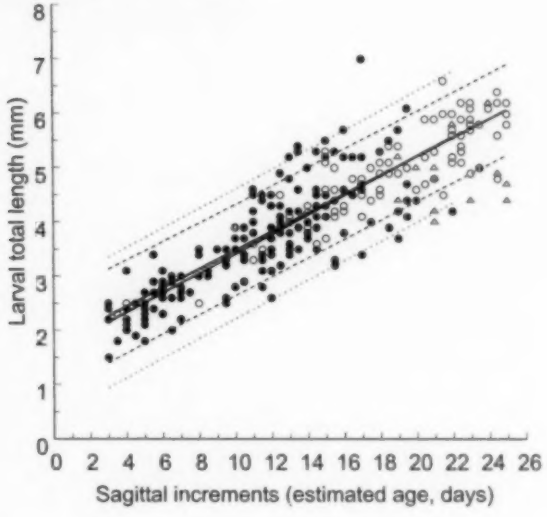
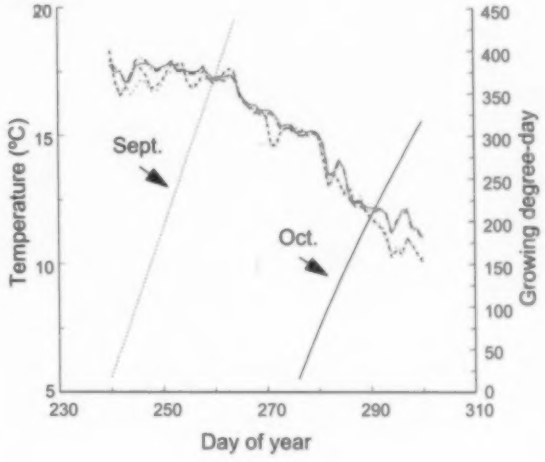


Fig. 5. Smoothed temperature time series for 28 August to 27 October 1997 (days 240–300) collected at two moorings on Western Bank at 2 m (dashed and dot-dashed lines) and 11 m (solid and dotted lines) depths. GDD trajectories for the September and October cohorts are shown as dotted and solid lines, respectively.



variation in the otolith–fish size relation may artificially create variation in AOGR that does not reflect that found in ASGR (AOGR = –23 + 1.7 × 10^3 ASGR; r^2 = 0.59). Thus, all analyses were performed using ASGR and AOGR.

The single physical variable that accounted for the most variance in ASGR in subcohort 1 was bottom depth (total depth of the water column at a given location; Table 2). Salinity averaged over the mixed layer explained the most variance in AOGR (Table 2). Multivariate analyses incorporating bottom depth, salinity, and larval silver hake concentration explained 60% of the variance in ASGR. The slope parameter for silver hake concentration was negative and had a marginal (<5%) but significant influence on the explained variance. Bottom depth, salinity, and hatch date explained 62% of the variance in AOGR.

Bottom depth and (or) salinity appear to account for a significant proportion of the variation in growth rate on Western Bank. In general, salinity and bottom depth are correlated because higher salinity water typically occurs over greater depths on the Scotian Shelf. Partial correlations between ASGR and bottom depth (salinity removed; Pearson r = 0.52, Spearman r = 0.46) and between ASGR and salinity (bottom depth removed; Pearson r = 0.25, Spearman r = 0.21) suggest that ASGR variation is better explained by bottom depth than by salinity, and bottom depth is a simple measure of geographic location on and around Western Bank. ASGR and AOGR calculated at 5-m bottom depth intervals increased by ~0.17 mm·d^{-1} and ~220 μm^2·d^{-1}, respectively, for larvae collected (over greater bottom depths) further from the crest of Western Bank (Fig. 6).

The rmMANOVA showed no difference in the trajectories of the back-calculated TL-at-age and daily growth rate series (p = 0.41 and 0.27, respectively) for the five larvae representative of each water mass on- and off-bank. However, when data for all 22 larvae were combined to define on- and off-bank TL-at-age and daily growth rate series, the slope for each series was greater for off-bank larvae than for on-bank larvae (ANCOVA, p < 0.001). Individual ANOVA at each increment using data for the 22 larvae indicated that the average back-calculated length and growth rate at each increment were not different (p > 0.005) between on- and off-bank larvae until at least 8 d had elapsed (Fig. 7). After this age, larvae collected off-bank were larger at a given age and had greater daily growth rates relative to larvae collected on-bank. In comparison, there was no difference in back-calculated TL at hatch between larvae collected on- and those collected off-bank (ANOVA, p = 0.27, N = 15).

Subcohort 2 (October)

Contrary to the pattern observed in the residuals of the September cohort, the residuals of the TL-at-age relation for the October cohort showed no pattern. The relation between ASGR and AOGR calculated using eqs. 1 and 2 for larvae in subcohort 2 was weak (AOGR = –7.7 + 7.7 × 10^2 ASGR; r^2 = 0.20), perhaps reflecting the nonlinearity in the otolith–fish size relation observed for larvae collected in October. Consequently, the results differed for ASGR and AOGR. A strong relation was apparent between AOGR and larval age, hatch date, and GDD (all interrelated variables), while these relations were weak for ASGR (Table 3). Bivariate relations with all other physical and biological variables were weak at best (Table 3). As date of collection was the same for all larvae in October, it is not included in the analyses. A

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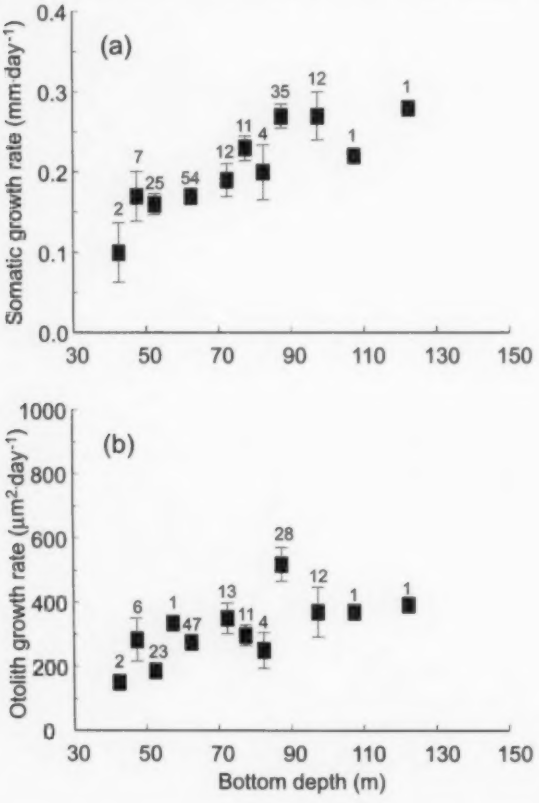
Table 2. Least squares regression statistics for the relations between ASGR (N = 164) and AOGR (N = 149) and all physical and biological variables examined for silver hake larvae aged 10–20 d collected on Western Bank in September 1997.

	ASGR			AOGR		
Variable	Slope	Intercept	r^2	Slope	Intercept	r^2
Bottom depth (m)	2.8×10^{-3}	3.8×10^{-3}	0.56**	5.3	–51	0.39**
Salinity (psu)	0.13	–4.0	0.43**	3.2×10^{2}	-9.8×10^{3}	0.45**
Density (kg·m^{-3})	0.13	-1.3×10^{2}	0.27**	2.0×10^{2}	-2.0×10^{5}	0.13**
Silver hake concentration (no.·m^{-3})	-4.2×10^{-3}	0.23	0.13**	–3.8	3.5×10^{2}	0.022
Larval concentration (no.·m^{-3})	-3.9×10^{-3}	0.23	0.12**	–3.2	3.5×10^{2}	0.016
Zooplankton biomass (g·m^{-3})	–0.18	0.23	0.053*	-2.1×10^{2}	3.6×10^{2}	0.015
Hatch date (d; day of year)	1.2×10^{-3}	-9.6×10^{-2}	<0.01	–14	3.8×10^{3}	0.093**
Age (d)	-2.7×10^{-4}	0.21	<0.01	16	95	0.091**
Temperature (°C)	8.7×10^{-3}	5.3×10^{-2}	<0.01	58	-6.6×10^{2}	0.085**
GDD (°C·d)	-2.1×10^{-5}	0.21	<0.01	0.92	87	0.088**
Mixed layer depth (m)	5.9×10^{-4}	0.19	<0.01	–5.5	4.2×10^{2}	0.028*
Collection date (d; day of year)	1.6×10^{-3}	–0.22	<0.01	–4.3	1.4×10^{3}	<0.01

Note: *$p < 0.05$; **$p < 0.001$.

Fig. 6. Scattergram of average ± 2 SE (*a*) somatic and (*b*) otolith growth rates for silver hake larvae aged 10–20 d collected over 5-m bottom depth intervals on Western Bank in September 1997. Numerals above error bars indicate sample sizes.



multivariate model with GDD and depth was able to explain only 4.3% of the variance in ASGR within subcohort 2, but a multivariate model with depth and age was able to explain 38% of the variance in AOGR, with only age making a significant contribution to the explained variance.

Discussion

Growth variation among monthly cohorts

The TL-at-age relations for September and October cohorts of larval silver hake on the Scotian Shelf were best described by linear least squares regression that revealed cohort-specific growth rates of 0.17 and 0.18 mm·d^{-1}, respectively. These are the first estimates of TL-at-age relations for silver hake larvae in the northwest Atlantic that we know of. The rates are similar to that of 0.16 mm·d^{-1} reported for Pacific hake (*M. productus*) larvae aged at <20 d (Bailey 1982) and are within the range of 0.135–0.279 mm·d^{-1} reported by Cass-Calay (1997) for Pacific hake larvae collected from environments with varying prey concentrations.

We did not expect virtually identical TL-at-age relations between September and October given that the GDD estimates for these cohorts differed by ~117°C·d (analogous to an average daily temperature difference of ~4°C) and that the zooplankton biomass estimate was about twofold greater for the October cohort than for the September cohort. We are confident of the temperature data, and our use of it, given the similarities in the time series from different depths in the surface mixed layer at different locations on Western Bank and the evidence that the larvae reside in the mixed layer (Fortier and Villeneuve 1996; C. Reiss, Department of Oceanography, Dalhousie University, Halifax, N.S., unpublished data). We are wary of our interpretations related to zooplankton biomass as an index of the larval prey field, as the collections were made with large and different mesh sizes and gear types for each cohort (333-µm-mesh BONGO for September, 243-µm-mesh BIONESS for October). However, Colton et al. (1980) found no difference in the displaced volume of plankton (positively correlated with biomass) collected with 253- and 333-µm-mesh nets at tow speeds similar to those used by us (0.5–1 m·s^{-1}). What is most important is our inability to distinguish between growth rates in cohorts that have experienced different ther-

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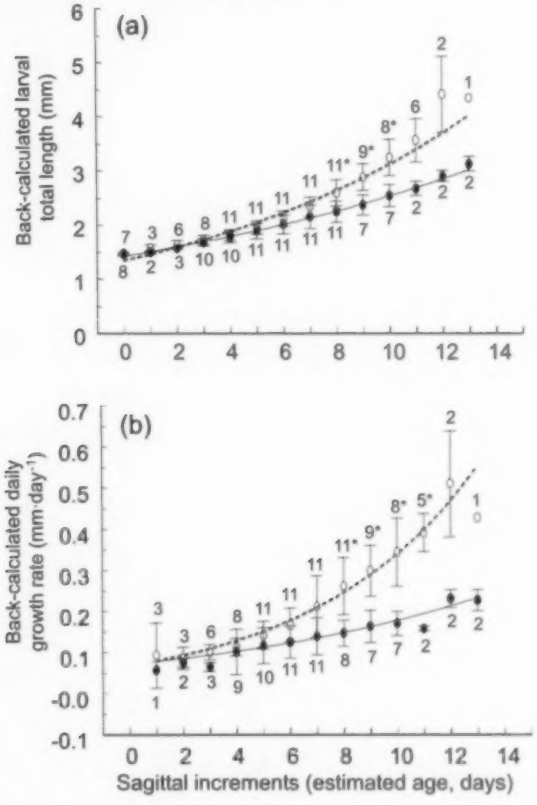
Fig. 7. Scattergram of average (*a*) TL and (*b*) daily growth rate series (back-calculated using surface area of each increment and the biological intercept model: Campana 1990) for silver hake larvae collected on and off Western Bank in September 1997. Relationships based on all estimates are shown by the dashed (off-bank) and solid (on-bank) lines: off-bank TL = $1.4e^{(0.084age)}$, $r^2 = 0.89$; off-bank GR = $0.066e^{(0.16age)}$, $r^2 = 0.77$; on-bank TL = $1.4e^{(0.058age)}$, $r^2 = 0.88$; and on-bank GR = $0.074e^{(0.090age)}$, $r^2 = 0.45$. Average ± 1 SD TL and daily growth rate estimates (off-bank, open circles; on-bank, solid circles) and sample sizes (off-bank, above; on-bank, below) are shown for each age. Significant differences (ANOVA, $p < 0.005$) at age between on- and off-bank larvae are indicated by an asterisk.



mal and feeding environments. Thus, neither temperature nor potential prey concentration alone can explain variation in growth between monthly cohorts, and perhaps a combination of these variables may be instructive (e.g., Rutherford et al. 1997; Gallego et al. 1999). Our initial premise that cohorts experiencing different environmental conditions will have different growth rates is invalid. Further, TL-at-age relations did not differ between larvae collected during day and night (in September 1997), and we can conclude that light-dependent gear avoidance (day–night effect) is insufficient to explain the similarity in TL-at-age among cohorts of similar age.

Strong relations were observed between otolith surface area and TL for larvae collected in September, October, and November, and the relations were different. This is consistent with others who have shown otolith–fish size relations to be dependent on temperature (Mosegaard et al. 1988) or prey concentration (Secor et al. 1989) through their influence on somatic growth rate (Secor et al. 1989). We reject the widely reported hypothesis that fast-growing larvae have smaller otoliths relative to body size than do slow-growing larvae (Secor et al. 1989), at least among monthly cohorts of silver hake larvae, as the TL-at-age relations were the same for September and October. However, we were unable to reject the same hypothesis within each monthly cohort, as a significant ($p < 0.002$) positive relations was observed between the residuals of the TL-at-age relation and the residuals of the otolith–fish size relations. Thus, the explanation for variation in the otolith–fish size relations among monthly cohorts is not easily determined using these data, but variation in the relation within cohorts can, in part, be explained by variation in growth rate. We must conclude that the relation between otolith and fish size in silver hake is not robust for larvae that have experienced a variety of environmental conditions. Otolith growth trajectories should, therefore, not be compared directly for larvae in different cohorts, and cohort-specific otolith–fish size models should be developed.

Our observation that TL-at-age was similar for cohorts experiencing different environments, however surprising, is not unique. Yoklavich and Bailey (1990) found no differences in growth rates of larvae collected in different years despite an average temperature difference of ~2°C. Furthermore, no relation was observed between growth rate and temperature in Pacific hake for temperatures between 10.5 and 12.4°C (Cass-Calay 1997). We hypothesize that the similarities in growth rate among cohorts may be explained by (1) variations in temperature and zooplankton biomass (potential prey) that were insufficient to produce a measurable difference in growth rate, (2) synergistic effects of temperature and zooplankton biomass on growth, and (3) enhanced prey requirements for larvae with increased metabolic rates related to higher water temperatures (Anderson 1988). Hypotheisis 1 is an unlikely explanation given the large ranges in temperature and prey levels experienced by the September and October cohorts. The other two hypotheses are not as easily dismissed. Larvae in the September cohort had a higher GDD history and nearly half the zooplankton biomass relative to those in the October cohort. Temperature and prey concentration may therefore have acted in concert to result in similar growth rates (hypothesis 2), or the lower potential prey levels in September may have been insufficient to meet the increased metabolic demand incurred by the higher GDD (hypothesis 3). It will be logistically difficult to distinguish between hypotheses 2 and 3 in field studies. However, the hypothesis that temperature and prey levels interact to determine TL-at-age relations in monthly cohorts of silver hake larvae can and should be tested using independent data derived from larval cohorts that have experienced different temperature and prey field histories.

The similarity in slopes of the TL-at-age relations for the September and October cohorts, despite different environmental conditions, indicates that aggregate estimates of larval growth at the monthly scale may be of limited value in predicting growth rate using environmental measures and

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Table 3. Least squares regression statistics for the relations between ASGR and AOGR and all physical and biological variables examined for silver hake larvae aged 10–20 d (N = 53) collected on Western Bank in October 1997.

	ASGR			AOGR		
Variable	Slope	Intercept	r^2	Slope	Intercept	r^2
Bottom depth (m)	-2.3×10^{-4}	0.19	0.040	0.19	1.2×10^{2}	<0.01
Salinity (psu)	2.8×10^{-2}	–0.70	0.045	16	-3.6×10^{2}	<0.01
Density ($kg \cdot m^{-3}$)	2.8×10^{-2}	–29	0.05	16	-1.6×10^{4}	<0.01
Hake concentration ($no. \cdot m^{-3}$)	2.9×10^{-2}	0.17	0.022	–48	1.4×10^{2}	0.021
Larval concentration ($no. \cdot m^{-3}$)	2.9×10^{-2}	0.17	0.023	–46	1.4×10^{2}	0.020
Zooplankton biomass ($g \cdot m^{-3}$)	1.2×10^{-2}	0.17	<0.01	–57	1.5×10^{2}	0.031
Hatch date (d; day of year)	6.3×10^{-4}	-1.4×10^{-3}	<0.01	–10	3.1×10^{3}	0.38**
Age (d)	-6.3×10^{-4}	0.19	<0.01	10	–34	0.38**
Temperature (°C)	-1.1×10^{-2}	0.30	0.023	–17	3.1×10^{2}	0.018
GDD (°C·d)	-5.1×10^{-5}	0.19	<0.01	0.80	–24	0.38**
Mixed layer depth (m)	5.6×10^{-4}	0.16	<0.01	–3.1	2.5×10^{2}	0.032

Note: $*p < 0.05$; $**p < 0.001$.

may not prove useful in the prediction of survival based on growth. Our suggestion is consistent with the idea that predicting growth, and ultimately survival, using environmental variables requires analysis at finer scales within cohorts and among individuals (Rice et al. 1993; Chambers and Miller 1995; Rutherford and Houde 1995). The results reported here benefit from a species and system that permit the finer scales of growth variability to be examined.

Growth variation within subcohorts

The residuals of the TL-at-age relations revealed growth to be a systematic function of geographic location within the September but not within the October cohort. This demonstrates the value of examining growth variation among months and cohorts, as conclusions based on results from one cannot be assumed to apply to another. The mostly positive residuals and higher ASGR and AOGR for larvae collected in deeper water off the crest of Western Bank during September indicate that larvae swept from the bank incur a growth advantage over larvae retained on the bank. Such an interpretation is consistent with Buckley and Lough (1987) and Frank and McRuer (1989) who observed that haddock (*Melanogrammus aeglefinus*) larvae in the shallow, mixed conditions on Georges Bank and Browns Bank, respectively, were in worse condition than larvae collected in deeper, stratified waters. Shackell et al. (1999) provided evidence that haddock advected from the Browns Bank region as larvae displayed greater size at age as juveniles than those presumed to have been retained as larvae on the bank. However, those same authors also provided evidence that the juveniles on Browns Bank had a lower overall mortality than those found elsewhere. Their findings are inconsistent with the hypothesis that slow growth is coupled with high mortality.

The variation in growth rates observed on- and off-bank indicates that growth is not homogeneous in the Western Bank region during late September at scales of ~15 km or less. This has serious implications for sampling and the estimation of population or cohort growth rates as they could be easily biased (high or low) depending on the water masses that represent the majority of the sampling. If growth estimates are biased, then survival estimates based on growth rate will be likewise.

We do not know what is directly responsible for the growth advantage observed for off-bank larvae. However, in this region the shelf break current is bathymetrically steered (Loder et al. 1997; Reiss et al. 2000) around Western Bank in an anticyclonic manner and larvae swept off-bank become entrained in a different water mass relative to those remaining on-bank. Possible explanations for the higher growth rates off-bank include (*i*) size-selective predation on- or off-bank, (*ii*) food limitation on-bank where larval concentrations are initially high, and (*iii*) a common origin for on- and off-bank larvae and enhanced growth for larvae transported off-bank. The similar variance in growth rates on- and off-bank and the negative relation between ASGR and larval concentration (silver hake or all species) are inconsistent with size-selective predation (hypothesis 1). The negative relation between ASGR and silver hake concentration does suggest that larvae on-bank may be food limited at high larval concentrations. No relation was observed between somatic growth rate and zooplankton biomass among locations on- and off-bank, and the results are therefore inconsistent with food limitation (hypothesis 2) and inferences that larval nutrition is inadequate off-bank (McLaren and Avedaño 1995). The analysis of individual growth trajectories for larvae collected on and off Western Bank indicates that the back-calculated TL-at-hatch was similar for all larvae and the average back-calculated TL-at-age and daily growth rate of larvae from the two water masses were similar until ~8 d posthatch. After this the larvae collected off-bank had a greater TL-at-age and a greater daily growth rate. Using the scaling arguments of Loder et al. (1988), the time scale for the along-bank drift was estimated as ~10 d using the average current speed (0.07 $m \cdot s^{-1}$, derived from the in situ current meters) and the along-bank length scale (60 km). This is of the same order as the observed divergence in growth rates of larvae on- and off-bank at 8 d posthatch. Thus, the variation in growth rates is most easily explained by the larvae having a common origin near the crest of Western Bank (consistent with O'Boyle et al. 1984) and those transported off-bank incur a growth advantage in the new water mass (hypothesis 3). We therefore hypothesize that growth variability within cohorts is determined by larval transport and the resulting water mass associations. Thus, the analyses of growth varia-

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tion in relation to physical oceanographic conditions will be required for predictions of larval growth variation in space.

The differences in average growth series on- and off-bank are not statistically supported by the rmMANOVA performed with five larvae from each water mass. This is related to the small sample size and the short time series (less than increment 10) that are a function of electing to measure the more conservative and less biased surface area of each otolith increment as opposed to increment widths along a single axis. The rmMANOVA results should thus be interpreted with caution, as the individual series extend over the first 10 d posthatch and the separation in growth trajectories determined at the population level only becomes apparent at 8 d. Furthermore, there is some uncertainty about the accuracy of daily otolith measurements for predicting daily somatic growth within individual larvae due to variation in the otolith–fish size relationships. However, in this study the somatic growth histories are back-calculated for relative comparisons among larvae in different water masses and the actual back-calculated sizes and growth rates are of little concern. We are confident that our results represent the relative differences in the daily growth series for larvae collected on- and off-bank.

In contrast with that observed in September, no clear relation was observed between growth rate and water mass structure, or geographic location, or any of the biological variables measured in October, perhaps due to the spatially constrained sample collections in October. We resampled the September collections in a manner that approximated the spatial distribution of the October collections to test this hypothesis. The results indicated that the proportion of the variance in growth rate explained by environmental variables (bottom depth, salinity, density, larval concentration, silver hake concentration, and zooplankton biomass) decreased when the September collections were spatially constrained. Therefore, we cannot reject the hypothesis that the lack of pattern in the October data is in part due to the spatially constrained samples collected during this period.

Based on hypotheses that suggest that larger or faster growing larvae have a survival advantage over smaller, slower growing larvae (Miller et al. 1988; Rice et al. 1993; Meekan and Fortier 1996), the results of this study suggest that survival should be greater for silver hake larvae that are removed from the crest of Western Bank early in development. However, as pointed out above, Shackell et al. (1999) gave evidence that this may not always be so. Clearly, examining larval growth and survival in relation to transport processes and at the scale of water masses within monthly cohorts may provide clarity and prove useful for predicting larval survival.

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DISCUSSION

Comments on "Contrasting patterns of productivity and survival rates for stream-type chinook salmon (*Oncorhynchus tshawytscha*) populations of the Snake and Columbia rivers" by Schaller et al. (1999)

Richard W. Zabel and John G. Williams

In a recent paper, Schaller et al. (1999) presented an analysis of spawner–recruit data that contrasts productivity patterns for stream-type chinook salmon (*Oncorhynchus tshawytscha*) stocks from the upper Columbia and Snake rivers (which must pass eight or nine dams) with those of stocks from the lower Columbia River (which must pass three or fewer dams). The main premise of the authors is that differences in productivity between upper and lower river stocks are primarily due to the number of dams that each must pass. In this comment, we examine three shortcomings of this analysis, shortcomings that we believe are severe enough to greatly weaken the conclusions of the paper.

First, we question the premise that the overriding difference between upper and lower river stocks is the number of dams that they pass. This premise assumes that upper and lower river stocks are similar enough that any differences in behavior, life history strategy, migration timing, age and size of migrant fish, and exposure to estuary and ocean conditions must be so small as to have no differential impact on these stocks. This is plainly not the case. Upper river and lower river stocks were placed in different evolutionarily significant units (ESUs) in consideration for listing under the U.S. Endangered Species Act because they were considered to be genetically different (Myers et al. 1998). In addition, upper river stocks must travel hundreds of kilometres farther than the lower river stocks. The demands of longer migrations alone would suggest a greater vulnerability to environmental fluctuations.

The same coded-wire tag data referred to by Schaller et al. (1999) provide evidence that upper river and lower river stocks behave differently in the ocean, contrary to the assertions of the authors. By extracting data from the Pacific States Marine Fisheries Commission Regional Mark Center (Table 1) for stream-type chinook salmon released from lower Columbia and Snake River hatcheries from brood years 1981 to 1988 (corresponding to the 1983–1990 migration years used by the authors), we can compare relative ocean recovery rates for these fish. Although, as a percentage of releases, ocean recovery rates for both groups were extremely low, 0.0003 and 0.0012, respectively, the difference between these rates was highly significant ($p < 0.001$; two-sample proportion test (Zar 1996, p. 552)). This fourfold higher ocean recovery rate for Snake River stocks as compared with lower Columbia River stocks suggests a difference in oceanic migration patterns or susceptibility to ocean harvest. Also, the majority of Snake River fish return as age-5 fish, while the majority of lower river fish return as age-4 fish (Beamesderfer et al. 1997). Given the differential ocean recovery rates and the differences in length of ocean residence, the regime shift to poor ocean conditions for West Coast Pacific salmon that occurred in 1976–1977 (Hare et al. 1999) could easily have impacted Snake River stocks more than lower Columbia River stocks and produced the contrasts in recruits per spawner observed by Schaller et al. (1999).

Second, even if we accept the premise that the only difference between stocks is the number of dams that they must pass, the data still do not provide clear evidence that dams are the sole cause of declines. Specifically, the authors argued that Snake and upper Columbia River survival rates exhibited a marked decline in the post-1974 brood years but lower Columbia River stocks did not. However, a careful inspection of the mean of log recruits per spawner (an index of survival) by region (Fig. 1) shows that data from upper Columbia River stocks do not support such a simple interpretation. In particular, the upper Columbia River stocks did not begin declining until the 1975 brood year (corresponding to the 1977 outmigration) or later. Juveniles from these stocks migrated downstream through a hydropower system that was completed in 1968, but their decline did not begin until approximately nine year-classes or nearly two generations later. The fact that these stocks did not begin to decline until well after the completion of the hydropower system suggests that other factors contributed to the decline. In light of this

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R.W. Zabel[1] **and J.G. Williams.** Northwest Fisheries Science Center, National Marine Fisheries Service, 2725 Montlake Blvd. E., Seattle, WA 98112, U.S.A.

[1]Author to whom all correspondence should be addressed.
e-mail: rich.zabel@noaa.gov

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Fig. 1. Mean ln(recruits/spawner) (an index of survival) for the Snake River (asterisks), upper Columbia River (triangles), and lower Columbia River (circles) chinook salmon stocks. Notice that the upper Columbia River stocks did not begin experiencing consistently depressed survivals until brood year 1975, corresponding to outmigration year 1977. The hydropower system was completed in 1968 in the upper Columbia River and in 1975 in the Snake River.

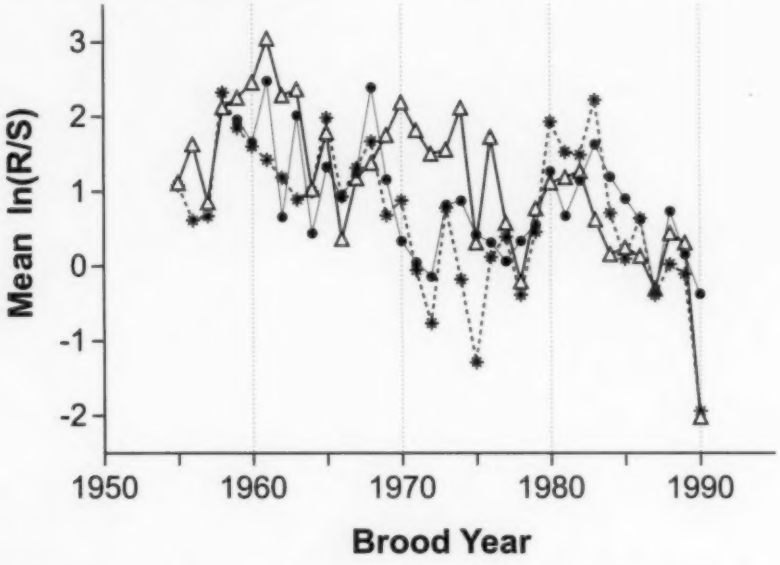

Table 1. Release and ocean recovery numbers of coded-wire-tagged stream-type chinook salmon released from hatcheries on tributaries of the Snake and lower Columbia Rivers.

	Lower Columbia River	Snake River
Number released	3 812 257	16 902 095
Expanded recoveries	11	211
% recovered	0.0003	0.0012

Note: The recoveries were expanded for sampling effort.

observation, the interpretation of the "late time period" for upper Columbia River stocks as a time period associated with hydropower-induced declines is suspect. A coincidence between stock declines and the completion of the hydropower system simply does not exist for these stocks.

Finally, the quality of the data used in the analysis is questionable. The reconstruction of stock information required many assumptions, and especially in the earlier periods, important information was lacking (Beamesderfer et al. 1997). In particular, age composition information on returning adults (i.e., the proportion of adults returning as 3-, 4-, 5-, or 6-year-olds) is not available during some time periods for some of the stocks. For example, prior to 1970, the early period of the comparison, no age composition data existed for any of the three lower river stocks or for the upper Columbia River stocks. In the post-1974 period, age composition data for the lower river stocks was available for 67% of the years. For the Snake River stocks, age composition data were available for the majority of years over the entire period. In years where no age composition data existed, average age compositions from available years were used (Beamesderfer et al. 1997).

Unfortunately, the lack of age composition data may strongly bias results in salmon stock recruit analyses when adults are assumed to return in constant age-class proportions each year. Bradford (1991) and Rivard (1989) concluded that age-class errors can decrease variability in reconstructed recruit time series. The net effect in the Ricker model used by Schaller et al. (1999) is that density dependence (b parameter) is overestimated, and since the a and b parameters are confounded, the a (productivity) parameter is also overestimated. Statistical analyses that compare productivities between periods and among regions that have inconsistent age composition data are thus inherently flawed. Although these limitations may not change the overall conclusions of the analysis, at the very least, readers should be informed of potential problems.

It is clear that hydroelectric development has played a role in the decline of Columbia and Snake River stocks. The analysis by Schaller et al. (1999) provides compelling arguments that dams are the overriding cause of the decline of upper river stocks, and hence that removing dams will restore salmon populations to historical levels. However, due to problems with the experimental design, shortcomings in the data used, and weakness in the assumption that different ESUs should respond identically to environmental change, these results do not, as Schaller et al. (1999) strongly concluded, rule out other factors contributing to the decline of these stocks. Other confounding factors, such as the increases in hatchery production and the implementation of a smolt transportation program, which were initiated in the late 1970s, render the situation even more complex. We are concerned that an overly simplistic interpretation of the data will lead to the neglect of other important factors, such as overfishing, habitat degradation (both freshwater and ma-

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rine), hatchery fish – wild fish interactions, and increases in exotic predators. Given the importance to the region of the question of whether or not to breach major dams, we feel that it is imperative that policy makers are provided with sound scientific information based on well-constructed analyses.

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Reply to Zabel and Williams' comments on "Contrasting patterns of productivity and survival rates for stream-type chinook salmon (*Oncorhynchus tshawytscha*) populations of the Snake and Columbia rivers" by Schaller et al. (1999)

Howard A Schaller, Charles E. Petrosky, and Olaf P. Langness

Introduction

In Schaller et al. (1999), we tested hypotheses about spatial and temporal patterns of productivity and survival rates for stream-type chinook salmon (*Oncorhynchus tshawytscha*) in relation to development and operation of the Federal Columbia River Power System. We discussed evidence that supported using these patterns to conclude that the dams had a major effect on the differential decline of upriver stocks. We found little evidence that hatchery, spawning, and rearing habitat or harvest factors could explain the spatial and temporal patterns of stock performance. The comments by Zabel and Williams (2000) do not alter our conclusions.

Zabel and Williams argue that there were three shortcomings of the Schaller et al. (1999) analyses, severe enough to greatly weaken the conclusion that differences in productivity between upper and lower river stocks are primarily due to the number of dams that each must pass. Zabel and Williams first question the premise that the overriding difference between upper and lower river stocks is the number of dams that they must pass, suggesting that different genetic background may predispose the upper stocks to different behaviors and greater vulnerability to environmental conditions independent of the hydrosystem. Second, Zabel and Williams take issue with our analysis of pattern of decline for the upper Columbia River stocks, which they assert did not decline until well after hydrosystem development. Their third point is that missing age composition data might bias the analysis. We address these three alleged shortcomings, and other misstatements about our conclusions, in the following sections.

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H.A Schaller.[1] U.S. Fish and Wildlife Service, 9317 NE Highway 99, Suite I, Vancouver, WA 98665, U.S.A.
C.E. Petrosky. Idaho Department of Fish and Game, 600 S. Walnut Avenue, P.O. Box 25, Boise, ID 83707, U.S.A.
O.P. Langness. Washington Department of Fish and Wildlife, 2108 Grand Boulevard, Vancouver, WA 98661, U.S.A.

[1]Author to whom all correspondence should be addressed.
e-mail: howard_schaller@fws.gov

Number of dams

Zabel and Williams question "the premise that the overriding difference between upper and lower river stocks is the number of that dams they pass." Schaller et al. (1999) did not claim that just the number of dams is the overriding factor. We specifically said that it was the effects of increasing hydropower development and operation that appeared to be extremely important in the decline and near extirpation of stream-type chinook salmon stocks of the upper Columbia and Snake rivers. We specifically said that the stocks from the three regions experienced different juvenile migration conditions due to mainstem hydropower development and operations. We further explained that effects included the direct effect of dams, decreased water velocity and increased migration time due to increased impoundment, and increased collection and transportation of juveniles (for the Snake River stocks) to circumvent mortality of juveniles. We explicitly said that we see little evidence of a systematic stressor that selected against upriver stocks, coincident with the declines and unrelated to the hydropower system. The count of number of dams oversimplifies the potential impact of hydropower development and operation. As an example, development of Canadian storage projects in the upper Columbia River in the mid-1970s and hydrosystem regulation has reduced flow during the smolt outmigration (Raymond 1988). This hydrosystem change would have begun to specifically affect upper Columbia River stream-type chinook salmon stocks in the mid-1970s. In addition, the number of turbine units that upper Columbia River stocks could encounter increased by as much as 43 units between 1965 and 1980 (Raymond 1988). Given that our hypothesis involved not just the number of dams, but effects of number of dams and operations, this claimed assumption would not weaken the conclusions of the paper.

Genetic differences

Zabel and Williams imply that there is a "weakness in the assumption that different ESUs [evolutionarily significant units] should respond identically to environmental change." Zabel and Williams hypothesize that genetic difference, and longer migration distances for upriver stocks, could explain

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the differential declines in stock performance. Zabel and Williams state that because these stocks were placed in different ESUs, one would expect upper and lower river stocks to respond differently to changing environmental conditions but provide no empirical evidence to support strong phenotypic differences among the ESUs. The technical document used for the ESU designation (Matthews and Waples 1991) was based primarily on genetic distance values computed for 21 polymorphic gene loci. Matthews and Waples (1991) showed that the estimated genetic distances of upstream and downstream stocks are small (and overlap in some cases). Myers et al. (1998) concluded that Snake River populations show a modest genetic difference in comparison with Columbia River stream-type populations. A minority of the Biological Review Team (for the Myers et al. 1998 report) felt that the ESU should be combined with the stream-type spring-run chinook salmon from the Columbia River.

The Schaller et al. (1999) hypothesis tests examined whether productivity and survival rate declined more and became more variable for upriver stocks, which were most affected by hydropower development, than for downriver stocks. The analysis did not assume that "different ESUs should respond identically to environmental change." The relevant issue is not whether there are any genetic differences between stock groupings, but whether genetic differences manifest themselves in a systematic change in stock performance coincident with and unrelated to hydrosystem development and operation. We presented evidence that the stock groupings had similar life history characteristics and behavior, and shared common environments, particularly during a critical period in the estuary and early ocean. We saw little evidence of a systematic stressor from climate influences or hatchery, spawning, and rearing habitat or harvest factors that could explain the observed spatial and temporal patterns.

To evaluate the claim that "longer upstream migrations alone would suggest a greater vulnerability to environmental fluctuations," we examined the long time series (1939–1990) of survival rate indices of the aggregate upriver spring chinook salmon run (Schaller et al. 1999). Patterns of the Pacific Decadal Oscillation and salmon production would indicate that poor ocean and climate conditions existed for Columbia River salmon before the late 1940s and after the late 1970s (Hare et al. 1999). Productivity and survival rates of the aggregate upriver spring chinook salmon run, which is heavily weighted by longer migrating Snake and upper Columbia River stocks, were relatively stable from 1939 until the era of major hydropower development when major declines began (fig. 7 in Schaller et al. 1999). If the populations had exhibited drops in survival rates of similar magnitude early in this time series, one could hypothesize that longer migration distances for upriver stocks in combination with the patterns of oceanic/climatic conditions may have systematically depressed upriver stocks coincident with, and unrelated to, development of the hydropower system. We believe that the evidence from the aggregate run does not support the theory that longer migration distances for upriver stocks could explain the differential declines in stock performance.

Schaller et al. (1999) reported evidence from the literature that common environmental processes influence survival rates of widely separated stocks within broad regions, particularly during the year of ocean entry. Common stock performance patterns were observed for Fraser River stream-type chinook salmon (Bradford 1994), Fraser River sockeye salmon (*Oncorhynchus nerka*), Bristol Bay sockeye salmon (Peterman et al. 1998), and these index stocks of Columbia River Basin stream-type chinook salmon (Deriso et al. 1996). In addition, climate patterns over the North Pacific Ocean may play an important role in determining abundance of different species of salmon originating over a wide geographic range (Beamish and Bouillon 1993).

Ocean recovery rates

Zabel and Williams state that the "same coded-wire tag data referred to by Schaller et al. (1999) provide evidence that upper river and lower river stocks behave differently in the ocean, contrary to the assertions of the authors." Zabel and Williams missed the major point that year-class strength appears to be strongly determined by the estuary and early ocean environments, where upriver and downriver stocks overlap in time and space. The sparse recovery data indicate general overlap of range from California to Alaska fisheries (Schaller et al. 1999). Even if later ocean distribution differs between stock groupings, which is not demonstrated clearly, the primary effect on survival rates would be expected earlier in the life cycle.

The coded-wire tag (CWT) data extracted by Zabel and Williams were not documented but clearly differed from data used in the Plan for Analyzing and Testing Hypotheses (PATH) that we referred to in Schaller et al. (1999). It is unclear which populations were sampled by Zabel and Williams and whether experimental and (or) production groups were included. For example, Zabel and Williams' data set had only 11 expanded recoveries for lower river stocks compared with 94 (41 observed) in the PATH data set.

Their conclusion about behavioral differences is particularly disturbing when one realizes that the recovery proportion differences are at the sixth decimal place. Even if one accepts the Zabel and Williams approach, it is consistent with our findings that all the stream-type chinook salmon harvest rates are less than 1%. This is in stark contrast with ocean-type chinook salmon, which experience harvest rates in the range of 30–40% (PSC-CTC 1988; Peters et al. 1999). Use of pooled release data, and expanded recoveries across years, may also be inappropriate when attempting to make inferences about behavioral changes in population distribution, since brood year strength could mask behavioral change.

The CWT data clearly indicate extremely low rates of ocean recovery for both groups. Inferences beyond this conclusion, such as differences in oceanic migration patterns, may not be warranted given the scarcity of data. The appropriateness of the two-sample proportion test used by Zabel and Williams for the CWT recovery rates is questionable. In fact, de Libero (1986) found, when evaluating aggregate CWT recovery data, that five or fewer *observed* recoveries are probably insufficient for stable estimates. Also, these data are predominated by zero mark recoveries in the ocean recovery strata. Therefore, a large sampling expansion factor applied to a single mark recovery could unduly influence the difference in ocean recovery rates between stock groups.

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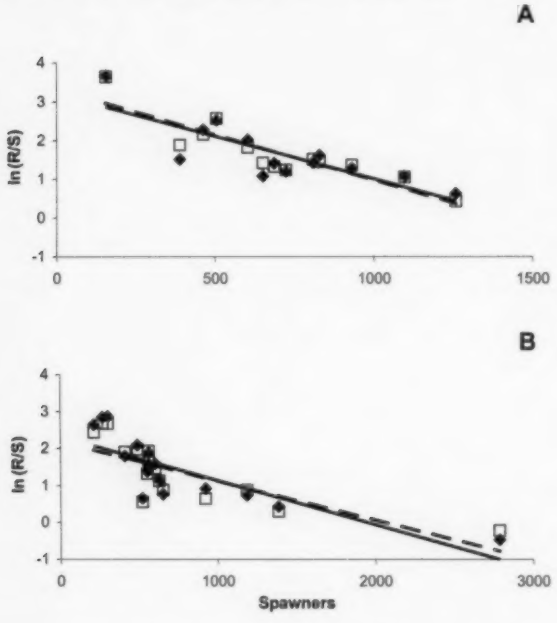
Fig. 1. Sensitivity to the effect of assuming a constant age structure on calculating recruits for pre-1970 return years for two of the Snake River index chinook salmon stocks: (A) Marsh Creek and (B) Minam River. The ln(*R*/*S*) is represented by symbols (diamonds, observed age; squares, constant age assumption) and the linear relationship of ln(*R*/*S*) to *S* is represented by lines (solid line, observed age; broken line, constant age assumption).



de Libero (1986) estimated that it takes between 40 and 50 observed recoveries to achieve a coefficient of variation of 20%. The expanded recoveries of 11, for the lower Columbia River group reported by Zabel and Williams, would represent four to five observed recoveries (applying the average expansion rate that we observed for this group in the PATH data set). According to de Libero's (1986) work, an observed tag number less than five would yield a coefficient of variation of greater than 70% and is probably insufficient for a stable estimate. Given the unstable nature of this estimate, and that the estimated differences in recovery rates are in the sixth decimal place, it appears presumptuous to conclude that this is empirical evidence "that upper river and lower river stocks behave differently in the ocean." Zabel and Williams further hypothesize that this unsubstantiated "behavioral difference" is due to genetic categorization of the upriver and downriver ESUs.

Longer life span

The majority of Snake River fish return as age-5 fish, while lower river stocks primarily return as age-4 fish. Zabel and Williams assert that given differential ocean recovery rates (both substantially less than 1%), this longer ocean residence could easily impact Snake River stocks more than lower river stocks and produce the observed contrasts in recruits per spawner. This conclusion is unsubstantiated and is not supported by the data. Presumably, a poor ocean environment would be especially influential on longer-lived stocks. There are two flaws in this conclusion. First, ocean survival rate patterns appear to be more strongly influenced in the first critical months in the estuary and early ocean environments (where the overlap is greatest between stock groupings; Pearcy 1992; Fryer and Schwartzberg 1993). Second, the adult age composition of the Minam River (in the Snake River) spring chinook salmon stock is younger and more similar to that of lower river stocks, yet the Minam River's stock decline was like that of other Snake River stocks (table 2 in Schaller et al. 1996).

Year of decline

Zabel and Williams argue that "a careful inspection of the mean of log recruits per spawner (an index of survival) by region ([their] Fig. 1) shows that data from upper Columbia River stocks do not support" that declines coincided with upper Columbia River hydrosystem completion in 1968. The log of recruits per spawner, ln(*R*/*S*), for those stocks did not begin to decline until brood year 1975. The hydropower system effects include more than just the number of dams. Effects include increased reservoir storage, reduced water velocity, delayed migration, and number of turbines encountered, all of which continued to change after 1968 (see Number of dams section). Zabel and Williams' reliance on ln(*R*/*S*) is problematic because they ignored density dependence. Recruits per spawner would tend to increase as spawners decrease, and the recruitment functions for the index stocks showed evidence of density dependence (table 2 in Schaller et al. 1999). The appropriate metric would be the deviations from the recruitment relationship (Peterman 1981; Hilborn and Walters 1992), which we termed survival rate indices (SRI-1 and SRI-2). Examination of the patterns of survival rate indices (fig. 5 in Schaller et al. 1999) does not clearly support Zabel and Williams' hypothesized lag in response. Survival rate indices suggest that the decline began between the mid-1960s and the mid-1970s for upper Columbia River stocks; noise in the data prohibits a precise determination of the first year of decline. The general pattern of decline for upper Columbia River stocks appears to be consistent with the observation that hydrosystem effects were in transition after the last dam was constructed (where flows decreased with increasing storage and number of turbines increased).

Age structure bias

Zabel and Williams express concern that lack of age data for lower and upper Columbia River stocks prior to 1970 may "strongly bias" results in the recruitment analysis. The net effect would be to overestimate density dependence (see above section) and productivity (Ricker *a* value). They state that statistical analysis of the effect of period and between regions is "inherently flawed," although limitations "may not change the overall conclusions of the analysis." We find it interesting that Zabel and Williams simultaneously question data quality and present arguments about specific years of decline for regional stock groupings. Although not discussed in Schaller et al. (1999), the missing age structure issue was addressed in a number of sensitivity analyses in the PATH process. PATH sensitivity analyses indicated that the recruit-

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ment functions, and simulated population performance, were relatively insensitive to these age structure assumptions (Deriso 1997).

We investigated the effect of assuming a constant age structure on calculating recruits for pre-1970 return years for two of the Snake River index stocks (Marsh Creek and Minam River), for which we had complete age structure estimates. We estimated the effect of this assumption on the Ricker *a* using the log transformation of our eq. 1 (Schaller et al. 1999) for the pre-1970 period. The effect on the Ricker *a* was small for both stocks (Fig. 1). The constant age structure assumption increased the Ricker *a* by 4% for Marsh Creek and decreased the Ricker *a* by 6% for the Minam River. These were very small changes compared with the total decrease in productivity observed between the pre-1970 and post-1974 periods (table 2 in Schaller et al. 1999). The overall conclusions of Schaller et al. (1999) appear to be robust to the age structure assumptions.

Conclusions

Zabel and Williams assert that problems with (*i*) experimental design, (*ii*) shortcomings in the data used, and (*iii*) weakness in the assumption that different ESUs should respond identically to environmental challenge do not allow Schaller et al. (1999) to rule out other factors contributing to the decline of these stocks. We believe that their assertion about the problem with experimental design is associated with comparing upriver and downriver population performance. This seems to be similar to their third argument that we should not assume an identical response by the different ESUs to a common set of environmental changes. Again, we did not assume that different ESUs should respond identically to environmental change. The relevant issue is not whether there are any genetic differences between stock groupings, but whether genetic differences manifest themselves in a systematic change in performance coincident with and unrelated to hydrosystem development and operation. The empirical evidence supporting behavioral differences in the ocean between upriver and downriver stocks (which are hypothesized to be from genetic differences) is extremely weak. If we assumed that genetic differences do cause a differential response to hydrosystem development and operation, we would still expect the response to be in a similar direction. Indeed, all Snake River anadromous salmonids have been listed under the Endangered Species Act or declared extinct (coho salmon (*Oncorhynchus kisutch*)) since completion of the hydropower system.

The assertion about shortcomings in data appears to revolve around missing age structure. We investigated the claim that bias due to a constant age structure assumption could invalidate our conclusions. Our sensitivity analysis showed that the effect of this assumption on the Ricker *a* was small, consistent with previous investigations in PATH. This finding provides evidence that the overall conclusions of Schaller et al. (1999) are robust to age structure assumptions. Zabel and Williams' final concern is that an overly simplistic interpretation of the data will lead to neglect of other important factors, such as overfishing, habitat degradation, hatchery fish – wild fish interactions, and increases in exotic predators.

We agree with Zabel and Williams that these other factors should not be neglected. Our approach was presented as a first step in a comprehensive decision analysis process. The coincidence in space and time of anthropogenic and environmental factors with the trend in spawner–recruit patterns lends credence to hypothetical assumptions about the importance of those factors in the decline of fish populations. The interpretations that we made were specific to stream-type chinook salmon of the Columbia and Snake rivers. Factors of decline might have different significance for other specific populations (for example, over harvest might be an important factor coincident with the decline of ocean-type chinook salmon from the Snake River). Our paper also emphasized the importance of assessing recovery actions in terms of smolt-to-adult and adult-to-adult survival rates.

We believe that the evidence that we presented and our reply to Zabel and Williams' comments show that we did not oversimplify interpretations of the data, which would "lead to the neglect of other important factors." We conclude that Zabel and Williams' comments do not warrant altering our conclusions in Schaller et al. (1999).

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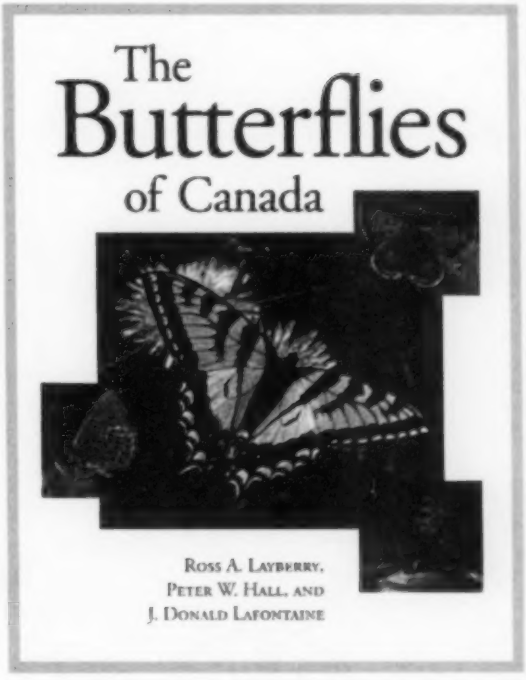

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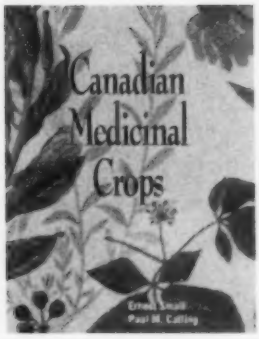

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